PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 11 September 1998 (11.09.98)	
International application No.: PCT/AU98/00149	Applicant's or agent's file reference: 1103PCT
International filing date: 06 March 1998 (06.03.98)	Priority date: 06 March 1997 (06.03.97)
Applicant: ROBERTSON, Sarah, Anne et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

24 August 1998 (24.08.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00149

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 38/18, 39/00, G01N 33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
AU:See search terms below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT, MEDLINE. SEARCH TERMS: INFERTIL, CONCEPTION, PREGNAN, FERTIL, ABORTION, MISCARRIAGE, PATERNAL, SPERM, MALE, TGF, TRANSFORMING GROWTH FACTOR, ACTIVIN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Journal of Clinical Immunology, Sept (1997) Vol 17(5) U.Gafter et. al. "Suppressed cell-mediated imunity and monocyte and natural killer cell activity following allogenic immunization". Pages 408-419, especially page 418, last paragraph.	1-49

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 MAY 1998	-2 JUN 1998
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer JAYNE BRITON Telephone No.: (02) 6283 2246

Form PCT/ISA/210 (second sheet) (July 1992) coplma

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00149

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Fertility and Sterility, vol 66(2) 1996, T.M.chu et. al. "Localisation of seminal plasma transforming growth factor - beta 1 on human spermatozoa: an immunocytochemical study." Pages 327 to 330, especially page 327 last paragraph, page 330 last paragraph.	1-49
X	American Journal of Reproductive Immunology, vol 33(4), 1995 M.Nocera and T.M.Chu "Characterisation of Latent Transforming Growth Factor - beta from human seminal plasma" pages 282 to 291. Whole document	1-49
X	WO 91/10445 (GENENTECH, INC) 25 July 1991 (For example, claim 1)	1
X	US 5395825 (YALE UNIVERSITY and TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 7 March 1995 (For example, column 3 line 66 to column 4 line 3, claims 3 and 4) (For example, column 3 lines 59 to 65, column 6 line 9 to 12 and line 23 to 26)	1 47
X, Y	US 5166190 (GENETECH, INC) 24 November 1992	46
Y	WO 95/04931 (SURFACE ACTIVE LIMITED) 16 February 1995	46, 47

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 98/00149

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9110445	AU	71734/91	EP	509040	WO	91/10445
US	5395825	AU	63998/94	CA	2156613	EP	688365
		WO	94/20637	US	5693479		
US	5166190	AU	71504/91	EP	510073	WO	91/10444
WO	95/04931	AU	73476/94	GB	9316369		

END OF ANNEX

AMENDED CLAIMS
[received by the International Bureau on 31 July 1998 (31.07.98); original claims 1-49 replaced by amended claims 1-48 (5 pages)]

1. A method of treating an infertility condition in a human or mammal by exposure of a prospective mother to one or more antigens of a prospective father and to substantially purified TGFβ or an effective derivative or analog thereof before attempted conception to elicit an immune reaction leading to tolerance to said one or more antigens to thereby alleviate symptoms of the infertility condition.

2. A method of treating an infertility condition as in claim 1 wherein a mucosal surface of the prospective mother is exposed to the one or more antigens.

3. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is selected from the group comprising an oral mucosal surface, a respiratory mucosal surface, a gastrointestinal mucosal surface or a genital mucosal surface.

4. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is a genital mucosal surface.

5. A method of treating an infertility condition as in claim 2 wherein the one or more antigens and TGFβ or derivative or analog thereof is injected for systemic contact.

6. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof and the one or more antigens are adminstered at one site.

7. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof and the one or more antigens are each adminstered at a first site and a different site respectively.

8. A method of treating an infertility condition as in claim 1 wherein the TGFβ or derivative or analog thereof and the one or more antigen are adminstered temporarily spaced apart.

9. A method of treating an infertility condition as in claim 8 wherein the one or more antigens are administered subsequent to administration of the TGFβ or derivative or analog thereof.

10. A method of treating an infertility condition as in claim 8 wherein the one or more antigens are adminstered first followed by administration of TGFβ or derivative or analog thereof.

11. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are chosen as a result of being particularly antigenic and prominent either on the sperm, or on the conceptus.

12. A method of treating an infertility condition as in claim 1 wherien the one or more antigens are present on cells taken from the prospective father that contain MHC antigens.

13. A method of treating an infertility condition as in claim 12 wherein the antigen is an MHC I antigen of the prospective father.

14. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are administered on leukocytes of the prospective father.

15. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are adminstrated on sperm cells of the prospective father.

16. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are adminstrated in the seminal plasma of the prospective father.

17. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are presented in purified or semi-purified form.

18. A method of treating an infertility condition as in claim 17 wherein the purified or semi purified one or more antigens are presented on inert or adjuvant carriers.

19. A method of treating an infertility condition as in claim 2 wherein humans are being treated, and the exposure of TGFβ is to a mucosal surface and the level of TGFβ is greater than 50 ng/ml with a total dose of 150ng/ml

20. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is exposed to a concentration of TGFβ of between 100 and 400ng/ml with a total dose of between 100 to 2000ng.

21. A method of treating an infertility condition as in claim 1 wherein the TGFβ or derivative or analog thereofis supplied in a slow release form.

22. A method of treating an infertility condition as in claim 1 wherein the exposure of the one or more antigens is to the prospective mother's genital tract in the form of the prospective father's ejaculate, and the level of exposure is determined by the cell count and antigenic density on the surface of such cells.

23. A method of treating an infertility condition as in claim 2 wherein humans are being treated and the one or more antigens are present on leukocytes, whereby between 10^7 and 10^9 leukocytes are adminstered to a mucosal surface.

24. A method of treating an infertility condition as in claim 1 wherein the TGFβ is selected from the group of $TGF\beta_1$, $TGF\beta_2$ and $TGF\beta_3$.

25. A method of treating an infertility condition as in claim 1 wherein the TGFβ is $TGF\beta_1$.

26. A method of treating an infertility condition as in claim 1 wherein the TGFβ is modified.

27. A method of treating an infertility condition as in claim 26 wherein the modification is selected from the group comprising substitution, deletion or addition mutants, peptide fragments of TGFβ or derivative or analog thereof, and peptide fragments of TGFβ or derivative or analog thereof which have been incorporated into another protein.

28. A method of treating an infertility condition as in claim 1 wherein the TGFβ or derivative or analog thereof is a member of the TGFβ superfamily.

29. A method of treating an infertility condition as in claim 28 wherein the member of the TGFβ superfamily is activin.

30. A method of treating an infertility condition as in claim 1 wherein TGFβ is administered in its active form.

31. A method of treating an infertility condition as in claim 1 wherein TGFβ is administered in precursor form.

32. A method of treating an infertility condition as in claim 1 wherein the prospective mother is incapable of converting sufficient of the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of active TGFβ.

33. A method of treating an infertility condition as in claim 1 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of a compound capable of activating TGFβ.

34. A method of treating an infertility condition as in claim 1 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of plasmin, so as to increase the level of active TGFβ.

35. A method of treating an infertility condition as in claim 1 wherein TGFβ is administered in an unpurified form using a biological source rich in TGFβ.

36. A method of treating an infertility condition as in claim 35 wherein the TGFβ is administered in the form of platelets.

37. A method of treating an infertility condition as in claim 2 wherein humans are being treated and the exposure to TGFβ and male antigen is a multiple exposure.

38. A method of treating an infertility condition as in claim 37 wherein the multiple exposure is preferably performed over a period spanning at least three months prior to attempted conception.

39. A method of treating an infertility condition as in claim 1 wherein humans are being treated and exposure is at least one week before conception is attempted.

40. A method of treating an infertility condition as in claim 1 wherein the exposure is before attempted conception

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference
International application No. PCT/AU98/00149	International filing date *(day/month/year)* 06-03-98	(Earliest) Priority date *(day/month/year)* 06-03-97

Title of invention
TREATMENT AND DIAGNOSIS OF AN INFERTILITY CONDITION

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Luminis Pty Ltd
1st Floor
10-20 Pulteney Street
Adelaide 5000
South Australia
Australia

Telephone No.: 08 83035020
Facsimile No.: 08 83034355
Teleprinter No.:

State *(that is, country)* of nationality: AUSTRALIA
State *(that is, country)* of residence: AUSTRALIA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ROBERTSON, Sarah Anne
24 St Peters Street
St Peters 5069
South Australia
Australia

(Applicant for the United States only)

State *(that is, country)* of nationality: AUSTRALIA
State *(that is, country)* of residence: AUSTRALIA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

TREMELLEN, Kelton Paul
3 Wallace Street
Vale Park 5081
South Australia
Australia

(Applicant for the United States only)

State *(that is, country)* of nationality: AUSTRALIA
State *(that is, country)* of residence: AUSTRALIA

☐ Further applicants are indicated on a continuation sheet.

See Notes to the demand form

International application No.
PCT/AU98/00149

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

A.P.T. Patent and Trade Mark Attorneys
GPO Box 772
ADELAIDE 5001
SOUTH AUSTRALIA
AUSTRALIA

Telephone No.: 08 84105040

Facsimile No.: 08 84105042

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[ ] the international application as originally filed

the description [X] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[X] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [X] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: .....

[ ] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Sheet No. 3

International application No.
PCT/AU98/00149

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:				For International Preliminary Examining Authority use only: received	For International Preliminary Examining Authority use only: not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:		sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	5	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	1	sheets	☐	☐
5. letter	:	1	sheets	☐	☐
6. other *(specify)*	:		sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Luminis Pty Ltd
By its Patent Attorneys
A.P.T. Patent and Trade Mark Attorneys

Paul Wyk Patent Attorney

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

PATENT COOPERATION TREATY

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:
A.P.T. PATENT AND TRADE MARK ATTORNEYS
G.P.O. Box 772
Adelaide, S.A. 5001
AUSTRALIE

Date of mailing (day/month/year) 11 September 1998 (11.09.98)	
Applicant's or agent's file reference 1103PCT	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/AU98/00149	06 March 1998 (06.03.98)	06 March 1997 (06.03.97)

Applicant
LUMINIS PTY. LTD. et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,BR,CA,CN,EP,IL,JP,KP,KR,NO,PL,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AL,AM,AP,AT,AZ,BA,BB,BG,BY,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,GM,GW,HU,ID,IS,KE,
   KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NZ,OA,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,
   TM,TR,TT,UA,UG,UZ,VN,YU,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   11 September 1998 (11.09.98) under No. WO 98/39021

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:
A.P.T. PATENT AND TRADE MARK ATTORNEYS
G.P.O. Box 772
Adelaide, S.A. 5001
AUSTRALIE

Date of mailing (day/month/year) 11 September 1998 (11.09.98)	
Applicant's or agent's file reference 1103PCT	**IMPORTANT INFORMATION**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/AU98/00149	06 March 1998 (06.03.98)	06 March 1997 (06.03.97)

Applicant
LUMINIS PTY. LTD. et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   AP :GH,GM,KE,LS,MW,SD,SZ,UG,ZW
   EP :AT,BE,CH,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,BR,CA,CN,CZ,DE,GB,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US,
   VN

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,ML,MR,NE,SN,TD,TG
   National :AL,AM,AT,AZ,BA,BB,BY,CH,CU,DK,EE,ES,FI,GE,GH,GM,GW,HU,ID,IS,KE,
   KG,KZ,LC,LK,LR,LS,LT,LU.LV,MD,MG,MK,MW,MX,PT,SD,SG,SI,SL,TJ,TM,TR,TT,UA,
   UG,UZ,YU,ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purposes of obtaining a European patent including, where applicable, ES which cannot be elected since it is not bound by Chapter II.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
J. Zahra

Telephone No. (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 1103PCT

Box No. I TITLE OF INVENTION

TREATMENT AND DIAGNOSIS OF AN INFERTILITY CONDITION

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

Luminis Pty Ltd
1st Floor
10-20 Pulteney Street
Adelaide SA 5000
Australia

☐ This person is also inventor.

Telephone No. 08 83035020

Facsimile No. 08 83034355

Teleprinter No.

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

ROBERTSON, Sarah Anne
24 St Peters Street
St Peters SA 5069
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

A.P.T. Patent and Trade Mark Attorneys
GPO Box 772
Adelaide SA 5001
AUSTRALIA

Telephone No. 08 84105040

Facsimile No. 08 84105042

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. ..2...

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

TREMELLEN, Kelton Paul
3 Wallace Street
Vale Park SA 5081
AUSTRALIA

This person is:

- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

See Notes to the request form

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AL Albania .....
☒ AM Armenia .....
☒ AT Austria .....
☒ AU Australia .....
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina .....
☒ BB Barbados
☒ BG Bulgaria .....
☒ BR Brazil .....
☒ BY Belarus
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China including Hong Kong
☒ CU Cuba .....
☒ CZ Czech Republic .....
☒ DE Germany .....
☒ DK Denmark .....
☒ EE Estonia .....
☒ ES Spain .....
☒ FI Finland .....
☒ GB United Kingdom
☒ GE Georgia .....
☒ GH Ghana .....
☒ GM Gambia
☒ GW Guinea-Bissau .....
☒ HU Hungary .....
☒ ID Indonesia
☒ IL Israel .....
☒ IS Iceland
☒ JP Japan .....
☒ KE Kenya .....
☒ KG Kyrgyzstan .....
☒ KP Democratic People's Republic of Korea .....
☒ KR Republic of Korea .....
☒ KZ Kazakhstan .....
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho .....
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MD Republic of Moldova .....
☒ MG Madagascar .....
☒ MK The former Yugoslav Republic of Macedonia .....
☒ MN Mongolia
☒ MW Malawi .....
☒ MX Mexico .....
☒ NO Norway
☒ NZ New Zealand .....
☒ PL Poland .....
☒ PT Portugal .....
☒ RO Romania
☒ RU Russian Federation .....
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia .....
☒ SK Slovakia .....
☒ SL Sierra Leone
☒ TJ Tajikistan .....
☒ TM Turkmenistan .....
☒ TR Turkey .....
☒ TT Trinidad and Tobago .....
☒ UA Ukraine .....
☒ UG Uganda .....
☒ US United States of America .....
☒ UZ Uzbekistan .....
☒ VN Viet Nam .....
☒ YU Yugoslavia .....
☒ ZW Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☐ .....
☐ .....
☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

See Notes to the request form

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1) AUSTRALIA	06/03/97	PO5508	AU
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present International application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA /

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	:	4 sheets
2. description	:	31 sheets
3. claims	:	5 sheets
4. abstract	:	1 sheets
5. drawings	:	6 sheets
Total	:	47 sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☐ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. 8 of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

For and on behalf of
Luminis Pty Ltd

Full Name: PETER ROBERTSON HART
Position Held: MANAGING DIRECTOR.

Sarah Anne Robertson

Kelton Paul Tremellen

For receiving Office use only

1. Date of actual receipt of the purported international application:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid

2. Drawings:
☐ received:
☐ not received:

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

See Notes to the request form

PATENT COOPERATION TREATY
PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1103 PCT : PJW : JWH : HJB	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 98/00149**	International filing date *(day/month/year)* 6 March 1998	Priority Date *(day/month/year)* 6 March 1997
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[6] A61K 38/18, 39/00, G01N 33/68		
Applicant (1) LUMINIS PTY LTD (2) ROBERTSON, S.A. et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **4** sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[X]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report 1 March 1999
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer **JAYNE BRITON** Telephone No. (02) 6283 2246

INTERNATIONAL PRI MIN[redacted]RY EXAMINATION REPORT

[redacted]rnational application No.
[redacted]T/AU 98/00149

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-31,** as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

☒ the claims, pages , as originally filed,
pages **32-35,** as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **41,** filed with the letter of **29 January 1999.**

☐ the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1-48**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-48**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-48**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

Claims 1 to 48 are inventive and involve an inventive step because there is no prior teaching in the art to treat infertility by exposing a prospective mother to paternal antigens and TGFβ or analogue.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Pages 36 to 40 are absent, because claim numbers 41 to 48 filed with letter on 29 January 1999, are on a page numbered 41.

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

A.P.T. Patent and Trade Mark Attorneys
GPO Box 772
ADELAIDE SA 5000

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *day/month/year*	13 OCT 1998
Applicant's or agent's file reference 1103 PCT: PJW: LKD	**REPLY DUE** within **three months** from the above date of mailing

International application No.	International filing date	Priority Date
PCT/AU 98/00149	6 March 1998	6 March 1997

International Patent Classification (IPC) or both national classification and IPC

Int. Cl.[6] A61K 38/18, 39/00, 601N 33/68

Applicant

(1) Luminis Pty Ltd
(2) Robertson, S. A. et al

1. This written opinion is the **first** drawn by this International Preliminary Examining Authority.
2. This opinion contains indications relating to the following items:.

I	[X]	Basis of the opinion
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is:

Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer **JAYNE BRITON** Telephone No. (02) 6283 2246

WRITTEN OPINION

ernational application No.
PCT/AU 98/00149

I. Basis of the opinion

1. This opinion has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

☐ the international application as originally filed.

☒ the description, pages **1-31,** as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

☒ the claims, Nos. , as originally filed,
Nos. **1-48,** as amended under Article 19,
Nos. , filed with the demand,
Nos. , filed with the letter of .

☐ the drawings, sheets/fig , as originally filed,
sheets/fig , filed with the demand,
sheets/fig , filed with the letter of .

2. The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig

3. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

WRITTEN OPINION

International application No.
PCT/AU 98/00149

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**17, 18, 45 and 48**	**YES**
	Claims	**1 to 16, 19 to 44, 46 and 47**	**NO**
Inventive step (IS)	Claims	**17, 18, 48**	**YES**
	Claims	**1 to 16, 19 to 47**	**NO**
Industrial applicability (IA)	Claims	**1-48**	**YES**
	Claims		**NO**

2. Citations and explanations

Citations are referred to by number, in the same order in which they are cited in the International search Report.

NOVELTY

Claims 1 to 16, 19 to 44 are not novel in light of citations 4, 5 and 6. Citation 5 discloses that "TGF beta may by administered prior to the introduction of ovum, sperm or conceptus into the reproductive tract of a female mammal". (Column 6 line 56 to 66). Citation 5 further discloses that the administration may by simultaneous with, prior to, or following, this. Citation 5 teaches the administration of TUF beta to a female followed by the administration of a paternal antigen (ie sperm). Thus the teaching of the citation 5 falls within the scope of the claims.

For similar reasons, the claims are not novel in light of citation 4. I note that your claim 28 defines "TGF beta or derivative or analog is a member of the TGF beta superfamily", and claim 29 which defines activin.

Claim 46 is not novel in light of citations 2, 3 or 5. For example, see citation 5, column 6 lines 7 to 26; or see citation 2, abstract conclusion; citation 3 abstract conclusion. Claim 47 is not novel in light of sperm and seminal fluid. Also not novel in light of citation 6.

INVENTIVE STEP

Claims 1 to 16, 19 to 44, 46 and 47 do not involve as inventive step for reasons give under novelty.

Claim 45 does not involve as inventive step in light of citations 2 and 3. These citations teach that the TGF beta in seminal plasma is related to fertility and "may immunologically protect the integrity of sperm". (Abstract) Knowing this, a person skilled in the art would be led to test the level of TGF beta in the sperm to diagnose infertility. Given that such assays are known (eg citation 7), this claim does not define an inventive step.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1103 PCT : PJW : JWH : HJB	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 98/00149**	International filing date *(day/month/year)* 6 March 1998	Priority Date *(day/month/year)* 6 March 1997

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[6] A61K 38/18, 39/00, G01N 33/68

Applicant
(1) LUMINIS PTY LTD
(2) ROBERTSON, S.A. et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **4** sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 6 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[X]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report 1 March 1999
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer **JAYNE BRITON** Telephone No. (02) 6283 2246

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU 98/00149

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-31,** as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

☒ the claims, pages , as originally filed,
pages **32-35,** as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **41,** filed with the letter of **29 January 1999.**

☐ the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement –

Novelty (N)	Claims	**1-48**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**1-48**	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	**1-48**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

Claims 1 to 48 are inventive and involve an inventive step because there is no prior teaching in the art to treat infertility by exposing a prospective mother to paternal antigens and TGFβ or analogue.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Pages 36 to 40 are absent, because claim numbers 41 to 48 filed with letter on 29 January 1999, are on a page numbered 41.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/18, 39/00, G01N 33/68

A1

(11) International Publication Number: WO 98/39021

(43) International Publication Date: 11 September 1998 (11.09.98)

(21) International Application Number: PCT/AU98/00149

(22) International Filing Date: 6 March 1998 (06.03.98)

(30) Priority Data:
PO 5508 6 March 1997 (06.03.97) AU

(71) Applicant *(for all designated States except US)*: LUMINIS PTY. LTD. [AU/AU]; 1sst floor, 10–20 Pulteney Street, Adelaide, S.A. 5000 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROBERTSON, Sarah, Anne [AU/AU]; 24 St. Peters Street, St. Peters, S.A. 5069 (AU). TREMELLEN, Kelton, Paul [AU/AU]; 3 Wallace Street, Vale Park, S.A. 5081 (AU).

(74) Agent: A.P.T. PATENT AND TRADE MARK ATTORNEYS; G.P.O. Box 772, Adelaide, S.A. 5001 (AU).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims and statement.

(54) Title: TREATMENT AND DIAGNOSIS OF INFERTILITY USING TGFβ OR ACTIVIN

(57) Abstract

A method of treating an infertility condition in humans or mammals, by exposure of a prospective mother to TGFβ or derivative or analog of TGFβ. The exposure is advantageously in conjunction with one or more antigens of a prospective father so that a hyporesponsive immune reaction is mounted to the one or more antigens of the prospective father.

FOR THE PURPOSES OF INFORMATION ONLY

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TREATMENT AND DIAGNOSIS OF INFERTILITY USING TGFβ OR ACTIVIN

FIELD OF THE INVENTION

This invention relates to a diagnostic method for an infertility condition giving rise to reduced ability to have offspring and to a method of treating such a condition.

BACKGROUND OF THE INVENTION

An inability or reduced ability to have children can cause great personal distress and has a high attendant social cost, particularly in terms of the cost of medical intervention. A large proportion of couples fall into this category. In the USA, for example, it is said that some 10-15% of couples of reproductive age are unable to have children, whereas in the United Kingdom this is 14%. In 1995 it was calculated that 5.1 million women had impaired fertility in the USA alone, with this figure projected to increase to 5.9 million by the year 2020 (56).In the US, the cost of a pregnancy conceived by IVF varies between US$66,000 for the first cycle to US$114,000 by the sixth cycle (60).

In the context of this patent an infertility condition is to be understood to relate not only the capacity to conceive but also to miscarriage, spontaneous abortion or other pregnancy related conditions, such as pre-eclampsia, and includes sub fertility.

Recent studies have revealed that a major proportion of infertile couples are childless because of a higher than normal rate of early embryonic loss (70% miscarriage v 21% miscarriage in fertile controls; 57), rather than an inability to conceive. These findings have initiated a search for reasons for the increased rate of early embryonic loss in infertile couples, as well as potential therapies to avert such losses.

In the last 20 years or so some hope has been held out to infertile couples with the development of *in vitro* fertilisation (IVF) techniques. These IVF techniques generally take the form of stimulating the female to ovulate, contacting collected ova with sperm *in vitro* and introducing fertilised ova into the uterus. Multiple variations of this general process also exist. Despite considerable research and technical advances in the IVF field the rate of successful pregnancy following IVF treatment is still quite low and is in the order of 15 to 25% per cycle.

Undertaking an IVF program often causes great anguish, especially when there is no resultant successful pregnancy. It is presently believed that the poor success rate in IVF treatment is due to an extraordinarily high rate of early embryonic loss (58, 59), possibly related to the patient's impaired reproductive state or the IVF process itself.

The low efficacy of IVF, together with its high cost and the associated psychological trauma from repeated treatment failures makes it desirable that alternative approaches to the problem of infertility are sought. Current methods of increasing pregnancy rates during IVF treatment include placing multiple embryos (2-5) into the uterine cavity, but this is not always effective since uterine receptivity is believed to be at fault at least as commonly as embryonic viability. Furthermore, the ensuing high rates of multiple pregnancy are associated with an increased maternal risk of pre-eclampsia, haemorrhage and operative delivery, and fetal risks including pre-term delivery with the attendant possibility of physical and mental handicap.

Similarly, early pregnancy loss is a major constraint in breeding programs for livestock and rare or threatened species. Embryonic mortality during the pre- and per-implantation period is viewed as the major reason for poor pregnancy outcome when assisted reproductive technologies such as artificial insemination are used. Even following natural mating, variability in litter size and in the viability of offspring are additional limitations with serious economic implications.

The reasons for increased rates of early embryonic loss following natural and assisted conception remain unknown. Chromosomal studies on miscarried embryos have confirmed that at least half of these embryos are genetically normal (61). Normal embryos appear to be lost primarily because the environment provided by the maternal tract during pre-implantation development or at the time of implantation into the endometrium is insufficient to nurture their growth and development. Embryos may lose viability or developmental potential if the maternal tract milieu comprises inappropriate or insufficient nutrients or peptide growth factors. Moreover, a primary determinant of uterine receptivity is provided by the maternal immune response to the conceptus, which is perceived as foreign or semi-allogeneic due to expression of both maternal and paternal antigens.

Medawar originally hypothesised that maternal immune accommodation of the semi-allogeneic conceptus may be facilitated by immunological tolerance to paternal transplantation antigens (major histocompatibility [MHC] antigens)(70). This hypothesis lost favour when it was found that pregnancy does not permanently alter the capacity of mice to reject paternal skin grafts (5, 46). However, the concept of transient hyporesponsiveness to paternal MHC antigens (46) is now receiving renewed attention, as a recent study by Tafuri *et al* (31) has provided clear evidence to show that during murine pregnancy, T-lymphocytes reactive with paternal class I MHC become 'anergic', or unable to recognise antigen due to internalisation of T-cell receptors. This

anergic state conferred 'tolerance' to paternal MHC antigen-expressing tumor cells, and was functionally operative from as early as implantation (day 4 of pregnancy) and lasted until shortly after parturition when lymphocytes regained their reactivity. The data support the hypothesis that a permissive maternal immune response to other antigens expressed on the embryo, or the fetal-placental unit (hereafter referred to as the conceptus) may similarly be due to induction of a tolerant immune response specific to those antigens.

Just precisely what is responsible for inducing this tolerance of paternal MHC antigens and other conceptus antigens has heretofore been unclear. Additionally the nature of the tolerance was unclear.

The term tolerance in the context of this invention is taken to mean inhibition of the potentially destructive cell-mediated immune response against conceptus antigens, and/or inhibition of synthesis of conceptus antigen-reactive immunoglobulin of complement-fixing isotypes (for example the 'Th1' compartment of the immune response). This tolerance may or may not be associated with induction of synthesis of non-destructive, conceptus antigen-reactive immunoglobulin of the non-complement-fixing isotypes and subclasses (for example the 'Th2' compartment of the immune response). The term tolerance should be taken to encompass T cell anergy and other permanent or transient forms of hypo-responsiveness or suppression of the maternal Th1 compartment.

Tafuri et al (31) have shown that paternal antigen-specific tolerance is active by the onset of blastocyst implantation on day 4 of pregnancy in mice. The pre-implantation embryo is a poor antigenic stimulus since it usually comprises fewer than 100 cells and is enveloped by a protective coat (zona pelluicida) until just before implantation. Semen however is richly endowed with paternal antigens present on and within sperm, somatic cells and the seminal plasma itself, and comprises an effective priming inoculum for many paternal antigens (5) known to be shared by the conceptus. Up until now seminal plasma has been conventionally thought to function primarily as a transport and survival medium for spermatozoa traversing the female reproductive tract (21). The recent studies described by the inventors in this specification have highlighted a hitherto unappreciated role for this fluid in interacting with maternal cells to induce a cascade of cellular and molecular events which ultimately lead to maternal immune tolerance to paternal antigens present in semen and shared by the conceptus, thereby abrogating immune rejection during implantation.

Ejaculation during coitus provokes a leukocyte infiltrate at the site of semen deposition termed the 'leukocytic cell reaction' in a variety of mammalian species, including man (1). In mice, the cascade of cellular and molecular changes initiated by the introduction of semen into the uterus, in many respects, resembles a classic inflammatory response. Within hours after mating, a striking influx and activation of macrophages, neutrophils, and eosinophils occurs in the endometrial stroma (2-4), in association with upregulated expression of major histocompatibility complex (MHC) class II and CD86 antigens by endometrial dendritic cells, followed by enlargement of draining lymph nodes (5,6). This inflammatory response is transient and fully dissipates by the time of embryo implantation on day 4 of pregnancy (2-4), when leukocytes persisting in the endometrium are predominantly macrophages with an immunosuppressive phenotype (7).

The temporal changes in trafficking and phenotypic behaviour of endometrial leukocytes during the period between mating and implantation are likely to be orchestrated principally by cytokines emanating from steroid hormone regulated epithelial cells lining the endometrial surface and comprising the endometrial glands (8). Of particular importance are granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-(IL)-6, the synthesis of which are upregulated at least 20-fold and 200-fold respectively in estrogen primed epithelial cells following induction by specific proteinaceous factors in seminal plasma (8,9) known to be derived from the seminal vesicle gland (10). Previous studies have implicated the surge in epithelial GM-CSF release as a key mediator in the post-mating inflammatory response since injection of recombinant GM-CSF into the estrous uterus is sufficient to produce cellular changes resembling those seen following natural mating (11). The inventors have found, using GM-CSF deficient mice, that the chemotactic activity of GM-CSF is likely to be compensated or augmented by an array of chemokines, the expression of which are transiently upregulated after mating (12), and cytokines synthesised by activated endometrial macrophages including IL-1 and tumour necrosis factor-α (TNF-α)(4).

The present inventors have investigated the nature of the seminal factor which acts to stimulate GM-CSF release from the uterine epithelium. Previous experiments have shown that the increase in uterine GM-CSF content is neither the result of introduction of GM-CSF contained within the ejaculate, nor a consequence of a neuroendocrine response to cervical stimulation, and is independent both of the presence of sperm in the ejaculate and MHC disparity between the male and female (8). A mechanism involving induction of GM-CSF mRNA synthesis in epithelial cells by proteinaceous factors derived from the seminal vesicle was suggested by experiments showing that

seminal vesicle-deficient (SV-) males did not evoke GM-CSF release or a post-mating inflammation-like response in females, and that trypsin-sensitive, high molecular weight material extracted from the seminal vesicle could upregulate GM-CSF release from uterine epithelial cells in vitro (10).

It has, however, not been clear from previously published work that this inflammatory response is related to the induction of tolerance by the mother to the conceptus, or alternatively whether the inflammatory response has a role in enhancing the immune system to combat the influx of foreign matter such as potential pathogenic bacteria is not clear. Nor is there any indication as to what the trigger for the induction of tolerance is or indeed that tolerance is mediated by semen.

One known relevant prior art document is United States patent specification 5395825 by Feinberg. This specification discloses a finding that suggests that elevated TGFβ in the female reproductive tract can facilitate production of fibronectin, a protein hypothesised to assist implantation by promoting adhesion of the embryo to the endometrial surface. The half life of TGFβ is only a few minutes and its effect on fibronectin is very short term. Therefore the administration of TGFβ in the above method can only be contemplated to assist implantation if delivered at precisely the time at which the pre-implantation embryo arrives in the uterine cavity. The present invention does not require such temporal precision in TGFβ delivery, nor does it purport that the effect of TGFβ is mediated through fibronectin.

SUMMARY OF THE INVENTION

The inventors have identified TGF-β as a principal immune regulatory molecule within seminal plasma. TGFβ produced in the latent form in the seminal vesicle gland is activated within the female reproductive tract where it acts to induce GM-CSF synthesis in uterine epithelial cells, thereby initiating the post-coital inflammatory response.

Additionally the inventors have shown that TGFβ, when administered to the female reproductive tract together with sperm or semen, can elicit tolerance towards male antigens, including paternal MHC class I antigens. This state of tolerance is evidenced by inhibition of Th1-type immune responses to paternal antigens, including delayed-type hypersensitivity (DTH) responses primed by a previous injection with sperm, production of complement-fixing isotypes of immunoglobulin specific for sperm, and cell-mediated immune rejection of tumor cells bearing the same MHC class I antigens as contained in the priming sperm inoculum. It is proposed that this tolerance might be achieved by exposure of the female to TGFβ either with or without male antigen.

The significance of this is that it is highly likely that certain infertility conditions will be related to the incapacity to produce tolerance to antigens of the male and/or to provide a suitable cytokine environment for growth and development of the pre-implantation embryo, as a result of either a lack of TGFβ in the seminal fluid of the male, an incapacity of the female to process the TGFβ from an inactive to an active form, or an absence or low levels of paternal antigens in the ejaculate. In some instances infertility may be due to the inability of the female to respond to TGFβ, in which case direct application of molecules induced by TGFβ, such as GM-CSF, may be warranted.

The TGF-β1 content of murine seminal vesicle secretions, like that of human seminal plasma (22), was found to be extraordinarily high and second only to that reported for platelet distillate (23). In mammalian species the TGF-β family comprises at least three closely related polypeptides, TGF-β_1, -β_2 and -β_3 (24), which exhibit 70-80 % sequence homology and share many biological actions. TGFβ_1 is the dominant TGFβ isotype responsible for increasing murine uterine GM-CSF output, since TGFβ_1-specific neutralising antibody is now found to have the ability to block 85% of seminal vesicle GM-CSF stimulating activity (Figure 2). Other members of the TGFβ superfamily, such as TGFβ_2 and activin, have also now been identified as capable of eliciting an increase in uterine GM-CSF output (Figure 4). These additional members of the TGF-β family, complexed with other carrier proteins such as the 250-300 kDa binding protein betaglycan (25) may account for the higher molecular weight activity present in murine seminal vesicle fluid and human seminal plasma (22).

The synthesis of TGFβ as a latent complex is believed to have a stabilising effect (26) and focus its activity at the target site by binding to extracellular matrix (27). Evidence for a uterine mechanism for activation of latent TGF-β was provided by the present finding that in contrast to activity in the seminal vesicle, the majority of the TGFβ_1 found in the uterine luminal fluid after mating was in the active form (Figure 5). Plasmin or other proteolytic enzymes derived from uterine cells or the male accessory glands (28, 29, 47) may contribute to the activation of TGFβ after ejaculation.

The proposal that components of the ejaculate can indirectly contribute to pregnancy success is supported by experiments in accessory gland-deficient mice (36, 37) and the finding that poor pregnancy outcome and dysregulated fetal and/or placental growth after embryo transfer or during first pregnancy in various livestock species (38-40) can be partially ameliorated by prior exposure to semen (41, 42). Likewise, studies in humans now clearly identify lack of exposure to semen due to limited sexual

experience, use of barrier methods of contraception, or in IVF pregnancies with increased risk of implantation failure, spontaneous abortion and pre-eclampsia (43-45).

In a broad form the invention could be said to reside in a method of treating an infertility condition in a human or mammal by exposure of the prospective mother to TGFβ or an effective derivative or analog thereof before attempted conception to elicit a transient hyporesponsive immune reaction to one or more antigen of a prospective father to thereby alleviate symptoms of the infertility condition.

In another broad form the invention could be said to reside in a method of treating an infertility condition in a human or mammal by exposure of a prospective mother to one or more antigens of a prospective father and to TGFβ or an effective derivative or analog thereof before attempted conception to elicit a transient hyporesponsive immune reaction to said one or more antigen to thereby alleviate symptoms of the infertility condition.

Preferably a mucosal surface of the prospective mother is exposed to the antigen, and more preferably the mucosal surface is the genital mucosal surface, however, it is feasible that exposure at other mucosal surfaces can give rise to the transient paternal antigen tolerance. There are two basic reasons that this might be the case, firstly it is known that tolerance to external antigens can be elicited at mucosal surfaces, thus it is known that women that are exposed to seminal fluid orally show evidence of reduced pre eclampsia effects to MHC antigens of the male partner (48). Thus the exposure could be oral, respiratory, gastrointestinal or genital. For example the surface antigen and TGFβ may be presented as an oral or nasal spray, or as a rectal or vaginal gel. Such a gel might for example be a gel such as used in the vaginal gel sold under the brand name PROSTIN (Upjohn Pty Ltd). Alternatively it might be desired to take the TGFβ and the surface antigen in a form that gives exposure to the small and perhaps large intestines, such as perhaps contained in a gelatin capsule.

Whilst a mucosal exposure may be preferred because it is likely to give rise to a transient tolerant immune reaction, it may also be feasible to provide for another route of exposure. Thus the surface antigen and TGFβ may be injected for systemic contact.

It may be desirable to deliver the TGFβ and the antigen together, for example where the two are combined in a gel, or spray, alternatively, it might be desirable to provide a source of TGFβ at the mucosal surface of interest, which might be the genital tract, and the antigen could subsequently be deposited onto the mucosal surface. It is also not yet

clear whether the TGFβ needs to be present at the same time as the antigen is present, although it is believed to be preferable, however, it is proposed that it may be possible to have a delay between the delivery of the TGFβ and the surface antigen. Thus an alternative would be to deposit the antigen first perhaps as an ejaculate and then deliver the TGFβ as a pessary after intercourse.

The nature of the relevant surface antigens is not entirely clear, but will presumably be those that are particularly antigenic and prominent either on the sperm, or on the conceptus. The most likely candidates are MHC antigens, and more preferably MHC class I. The most efficient manner of presenting these antigens is in the form that they are naturally present - on any appropriate cell of the intended male parent that expresses them and those cells would include sperm cells and may include leukocytes. The antigens may also be presented in biological fluids such as seminal plasma which is known to carry certain male antigens (49). This use of cells other than sperm cells will be pertinent where the sperm count of the prospective father is somewhat low. The use of cells other than sperm cells may be preferred where a non-genital route is used. Alternatively the antigens may be presented in purified or semi-purified form, which may or may not be presented on inert or adjuvant carriers, thus for example it may be presented in the carriers known as ISCOMS. This latter approach however is likely to be more technically complex and expensive. It is additionally possible that the antigens may be encoded within sperm cells in the form of mRNA (or other nucleic acid) and this RNA message is then expressed by maternal genital tract cells. It may be that TGFβ therefore plays a role in promoting the events leading to presentation of paternal antigen to maternal lymphocytes through activating genital tract antigen presenting cells to take up and translate sperm mRNA.

The level of TGF β may be varied, and will vary depending upon which species is being treated. For humans the level of TGFβ will preferably be greater than 50 ng/ml with a total dose of 150ng/ml and more preferably at a concentration of between 100 and 400ng/ml with a total dose of between 100 to 2000ng. The level of TGFβ in normal male semen is in the order of 200ng/ml. This level can be judged empirically when assessing other animals, and thus for horses or cattle the preferred level is expected to be in the order of 100ng/ml. These levels may vary if the TGFβ is supplied in a slow release depot, perhaps as a patch or as a gel or latent TGFβ complex.

The level of exposure to surface antigens may vary, in a preferred form the exposure will be to the prospective mother's genital tract in the form of the prospective father's ejaculate, and the level of exposure will be determined by the cell count and antigenic

density on the surface of such cells. Where cells are administered other than in the above manner, a similar number of cells might be used, however, the most effective manner may be determined empirically. It is though that an exposure of leukocytes in the order of 10^7 - 10^9 cells might be the appropriate level of exposure to a mucosal surface.

The specificity of TGFβ to be co-administered with the male antigens is at present not entirely clear, and because $TGF\beta_1$ is thought to be responsible whereas $TGF\beta_{2,3}$ are less important, it is more likely that $TGF\beta_1$ is to be used. It will however also be understood that various modification might be made to $TGF\beta_1$ or indeed $TGF\beta_2$, or $TGF\beta_3$ which could be effective in eliciting an effective transient tolerant immune reaction either separately or in combination with another agent. Such modified TGFβ's might include substitution, deletion or addition mutants, and might include peptide fragments, which may or may not be incorporated into another protein to make a recombinant protein. Alternatively other members of the TGFβ superfamily may also be used or used as a starting point to developing an analog of the TGFβ activity, one such member is known as activin.

Where unmodified TGFβ is used it will preferably be administered as $TGF\beta_1$. The $TGF\beta_1$ may be administered in its active form, however, where the prospective mother is capable of activating $TGF\beta_1$ it may also be administered in its precursor form. An alternative "delivery" option would be natural TGFβ such as in the form of platelets. Thus instead of purified TGFβ a preparation of platelets or other source rich in natural TGFβ, such as milk or colostrum, may be used.

The exposure is preferably a multiple exposure. The multiple exposure is preferably performed over a period of at least three months, with the mucosal surface being exposed to TGFβ during each exposure to the prospective father's antigens. This period of time could however be somewhat reduced, and it may be possible to achieve improvement with one exposure but as a minimum it is anticipated that exposure would be at least one week before conception is attempted. It may also be preferred that non-barrier contraceptive measures be taken prior to the planned conception, where the antigens are associated with sperm cells and these are administered to the genital tract, so that there is some certainty of a period of exposure to the prospective father's antigens before conception. This is particularly the case where the fertility condition is of the type where conception takes place but either miscarriage, spontaneous abortion or pre-eclampsia occurs after conception.

It is also envisaged that the administration of TGFβ in the presence or absence of the at least one surface antigen may need to continue past the prospective date of conception perhaps for the first 12 weeks of pregnancy.

In an alternative form the invention could be said to reside in a method of diagnosing an infertility condition in males by testing the level of TGFβ in seminal fluid.

Greater than 70% of the TGF-β_1 in seminal vesicles exists in the latent form. The infertility condition might therefore not be due to a lack of TGFβ in the semen of the male partner but it may be that the female cannot process the inactive form of the TGFβ. The invention could therefore also be said to include the method of exposing inactive form of TGFβ to the genital tract of a female and testing for her capacity to convert the inactive form of TGFβ to active TGFβ. If this is found to be the case, the method of treating the fertility condition will include administration of active TGFβ, or alternatively a compound capable of activating TGFβ can be administered, such as plasmin, so as to increase the level of active TGFβ.

In a preferred form the method of treating infertility will first include the step or diagnosing or testing whether the male has adequate levels of TGFβ or the female has the capacity to activate TGFβ, or alternatively whether anti-sperm antibodies exist.

The use of the present invention may be used in conjunction with IVF treatment, whereby the transient tolerant immune response is elicited before transfer of the conceptus or gametes is attempted. It is expected however that where the infertility condition is caused as a result of reduced TGFβ level in semen, or capacity to activate TGFβ, it is likely that the trauma of IVF treatment may not be needed and that a 'natural' conception may be possible in its place.

It will be understood that this invention is not necessarily limited to humans, but may also extend to treatment of other mammals including livestock species.

Some specific disorders or procedures that may benefit from the present invention are now discussed to some degree.

Recurrent miscarriage. It is known that approximately 2 -5 % of couples are involuntarily childless due to recurrent miscarriage. The aetiology of recurrent miscarriage is complex, but in the vast majority of cases no chromosomal, hormonal nor anatomical defect can be found and an immunological lesion is implicated. A

variety of therapies which attempt to modify the mother's immune response to the semi-allogeneic conceptus have been trialed with variable success. The predominant therapeutic approach over the past 20 years has been to inject women with paternal leucocytes in the hope of achieving 'tolerance' to paternal antigens. This therapy has had limited success with a meta-analysis of 15 trials concluding that paternal leucocyte immunisation can increase pregnancy rates by 8 - 10 % (51).

Coulam & Stern (52) have administered seminal plasma from a pooled donor source to the genital tract of women with recurrent miscarriage and were able to produce a non-statistically significant increase in live birth rates (60%v 48 %, p=0.29 n= 86). This treatment differs significantly from a preferred therapeutic regime in that seminal plasma was administered in the absence of paternal antigen. It is not surprising that the success of this therapy was limited, since no paternal antigen was administered.

The data supporting the present invention provide encouraging results which indicate that TGFβ may be a beneficial treatment for recurrent miscarriage because of its potent immune modulating capacity. It is expected that administration of sperm in combination with TGFβ will help produce a tolerant or 'nurturing' immune response to a future conceptus which would share some of the same MHC class I or other antigens.

Adjunct to IVF treatment. It is currently believed that premenstrual pregnancy wastage produces a significant negative contribution to IVF success rates. One theory for this increased early pregnancy loss is that IVF is an 'unnatural' process that separates the act of intercourse from conception. This would mean that IVF recipients may not be exposed to seminal plasma and it's associated antigens early in pregnancy. Several animal studies and human investigations, including the randomised control trial described herein, have suggested that exposure of the female genital tract to semen at the initiation of a pregnancy, as well as prior to a pregnancy, is beneficial to subsequent pregnancy outcome. It is proposed that there will be some benefit derived from giving women exogenous TGFβ in combination with partner's sperm/leucocytes at or near the time of embryo transfer, especially if the partner's seminal plasma TGFβ content is low or sperm numbers are low.

Anti-sperm antibody therapy. A significant proportion of infertility is due to the presence of anti-sperm antibodies in either the male or female partner (53). Seminal plasma has been shown to suppress the formation of anti-sperm antibodies in the female serum and genital tract secretions of the mouse. One of the active agents within seminal plasma responsible for suppressing maternal production of potentially

damaging, complement-fixing isotypes or subclasses of immunoglobulin specific for sperm antigens has been identified as TGFβ. It is expected that the present invention may, in at least some instances, block anti-sperm antibody formation. The relationship between maternal anti-sperm antibody formation in women and their partner's seminal plasma TGFβ concentration will be investigated to confirm this. Current therapies for anti-sperm antibodies are not sufficiently effective (for example oral steroids or the prolonged use of barrier contraception) or require expensive assisted reproduction therapy. It is proposed that administration of a TGFβ-containing pessary following intercourse will abrogate this anti-sperm antibody response and enable natural pregnancy to ensue.

Pre-eclampsia and IUGR prophylaxis. Pre-eclampsia and some forms of intra-uterine growth restriction (IUGR) are believed to be an immunological disorder due to 'shallow' placentation resulting from a damaging, Th1-type immune attack on the invasive trophoblast. There is epidemiological evidence showing that repeated exposure of a woman to her partner's antigens through intercourse in the absence of barrier contraception decreases her chances of developing pre-eclampsia in a subsequent pregnancy to that partner (54, 55). This may be brought about by the generation of maternal 'tolerance' towards paternal antigens as a consequence of repeated exposure at intercourse, which facilitates placental growth and invasion of the maternal decidua. Some women have a propensity to develop pre-eclampsia or to suffer fetal growth restriction every time they become pregnant. This may be due to inadequate TGFβ content of their partner's semen, or an inability to process latent TGFβ into a biologically active form.

Priming with partner's antigens in combination with TGFβ before conception and perhaps until 3 months of pregnancy, by which time placental invasion is complete, may help prevent the development of pre-eclampsia and IUGR in these high risk women.

Prospective analysis of stud animal fertility in livestock breeding industries. Variability in the productivity of stud males is a major constraint in pig, cattle, sheep and other livestock breeding programs. In many species there are substantial differences between studs, particularly in the pre-implantation mortality of embryos sired, even within a given herd. Currently, reliable estimation of the fertility and fecundity of a stud male is only possible after documentation of the outcome of multiple pregnancies. Measurement of the TGFβ content of seminal plasma of potential studs, for example by simple enzyme-linked immunosorbent assay, is likely to be an effective tool in livestock

breeding management. Such measurements may need to be taken over the course of some weeks and could be made in conjunction with measurements of other factors known to inhibit the action of TGFβ, such as interferon-γ.

Optimisation of pregnancy outcome in livestock breeding industries. A primary determinant of the productivity of livestock breeding programs, particularly in species such as the pig where litters are large, is variability in the litter size and weight of offspring. As detailed above, these parameters are believed to be influenced largely by the extent to which the mother's immune response is 'tolerised' to paternal antigens shared by the conceptus. Pregnancy outcome is often further compromised where the pregnancy is initiated by artificial insemination, particularly when artificial semen extenders, as opposed to seminal plasma, are employed as the carrier. Since the frequency of mating between breeding females and studs is often limited, and variability in the seminal plasma TGFβ content between males is probable, pregnancy outcome is likely to benefit from exogenous administration of TGFβ in many livestock species. TGFβ could be given prior to, or at the initiation of a naturally-sired pregnancy, or at the time of artificial insemination.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Sephacryl S-400 size exclusion chromatography of (*A*) GM-CSF stimulating activity and (*B*) TGF-β immunoactivity in murine seminal vesicle fluid. In *A*, uterine epithelial cells from estrous mice were incubated for 16 h with untreated (o, = active TGF-β) or acid activated (• = active + latent TGF-β) fractions of seminal vesicle fluid. After a further 24 h culture, the GM-CSF content of supernatants was determined by FD 5/12 bioassay. Values are means of triplicate cultures and the horizontal dashed line is GM-CSF production by epithelial cells cultured with DMEM-FCS alone. In *B*, the content of immunoactive TGF-β_1 (•) in fractions of seminal vesicle fluid was determined by ELISA. TGF-β bioactivity was detected by Mv-1-Lu cell bioassay. Fractions depicted by the hatched area contained > 300 pg / ml, and other fractions contained < 50 pg /ml. Data is representative of similar results obtained from three replicate experiments.

Figure 2. The effect of neutralising antibodies specific for TGF-$\beta_{1,2,3}$ and TGF-β_1 on GM-CSF stimulating activity in murine seminal vesicle

fluid. Uterine epithelial cells from estrous mice were incubated for 16 h with 2% crude seminal vesicle fluid or DMEM-FCS alone, in the presence or absence of mouse anti-bovine TGF-$\beta_{1,2,3}$ (20 µg / ml) or chicken anti-bovine TGF-β_1 (10 µg / ml). After a further 24 h culture, the GM-CSF content of supernatants was determined by FD 5/12 bioassay. Values are mean ± SD of triplicate cultures. Data is representative of similar results obtained from three replicate experiments.

Figure 3. The effect of TGF-β_1 on GM-CSF production by uterine epithelial cells in vitro. Uterine epithelial cells from estrous mice were incubated for 16 h with 0.08 - 80 ng / ml recombinant human TGF-β_1. After a further 24 h culture, the GM-CSF content of supernatants was determined by FD 5/12 bioassay. The mean ± SD of triplicate wells is shown. Data is representative of similar results obtained from four replicate experiments.

Figure 4. The effect of TGF-β_2, activin and inhibin on GM-CSF production by uterine epithelial cells in vitro. Uterine epithelial cells from estrous mice were incubated for 16 h with 0.05 - 50 ng / ml recombinant human TGF-β_1, porcine TGFβ_2, or human recombinant activin and inhibin. After a further 24 h culture, the GM-CSF content of supernatants was determined by FD 5/12 bioassay. The mean ± SD of triplicate wells is shown. Data is representative of similar results obtained from two replicate experiments.

Figure 5. The effect of seminal composition on the TGF-β_1 content of uterine luminal fluid after mating. TGF-β_1 immunoactivity was determined by ELISA in untreated (o = active TGF-β) or acid activated (• = active + latent TGF-β) uterine luminal fluids collected from estrous mice, or from mice 1 h after mating with intact, vasectomized (vas) or seminal vesicle deficient (SV-) males. Symbols represent data from individual mice and median values for treatment groups are scored. Data were compared by Kruskal-Wallis one way ANOVA and Mann Whitney Rank Sum test. Data sets labelled on the x-axis with different lower case letters denote statistical significance between treatment groups ($p < 0.01$).

Figure 6. The effect of intra-uterine TGF-β_1 on the GM-CSF content of uterine luminal fluid. Fluids were collected 16 h after natural mating with intact males, or after administration of 0.4 - 40 ng recombinant human TGF-β_1 in 50 µl PBS / 1% BSA, or vehicle only, to the uterine luminal cavity of estrous mice. Symbols represent data from individual mice and median values for treatment groups are scored. Data were compared by Kruskal-Wallis one way ANOVA and Mann Whitney Rank Sum test. Data sets labelled on the x-axis with different lower case letters denote statistical significance between treatment groups ($p < 0.01$).

Figure 7. The effect of rTGFβ_1 and semen on GM-CSF output from human reproductive tract epithelial cells. The GM-CSF content of culture supernatants collected from (A) cervical keratinocytes and (B) endometrial cell cultures was determined by commercial ELISA, 12 hours after the addition of dilute whole semen (10% vol/vol) or 10 ng/ml rTGFβ_1.

Figure 8. The effect of intra-uterine priming with sperm and TGFβ on induction of Th1-type immunity. Balb/c F1 female mice were immunised by intra-uterine infusion with CBA sperm in the presence of absence of 10ng rTGFβ_1. Additional groups of uterine-ligated mice were mated naturally with CBA males, or were given sub-cutaneous immunisations with sperm in complete Freund's adjuvant. Ten days later mice were assessed for DTH to sperm antigens, or serum content of anti-sperm IgG2b immunoglobulin. Data was compared by Kruskal-Wallis one way ANOVA, followed by Mann Whitney rank sum test with different superscripts indicating significant differences ($p < 0.05$).

Figure. 9. Effect of prior immunisation with sperm and TGFβ on fetal and placental weights during subsequent pregnancy in mice. Balb/cF1 female mice were immunised by intra-uterine infusion with CBA sperm in the presence ($\pm$ rTGFβ_1), and were mated naturally with CBA males 2 weeks later. Females were sacrificed on day 17 of pregnancy and fetal (A) and placental weights (B) were determined.

Comparisons between groups were made according to the number of viable fetal-placental units per uterine horn, by Kruskal Wallis one-way ANOVA followed by Mann Whitney rank sum test ($p < 0.05$).

DETAILED DESCRIPTION OF THE INVENTION

MATERIALS AND METHODS

Cell Lines, Media, Cytokines and Antibodies.

RPMI-1640 and low glucose Dulbecco's modified Eagle' medium (DMEM, GIBCO) were supplemented with 10% fetal calf serum (CSL), 20 mM HEPES pH 7.2, 5 $x10^{-5}$ M β-mercaptoethanol, 2 mM L-glutamine and antibiotics (RPMI-FCS and DMEM-FCS). FD5/12 cells (14), 3T3 fibroblasts, and JR-5 Balb/c fibrosarcoma cells were cultured in RPMI-FCS and mink lung cells [Mv-1-Lu, CCL-64] and uterine epithelial cells were cultured in DMEM-FCS. Human ectocervical cells were cultured in 70% DMEM, 20% Hams F-12 (Gibco), 9% FCS, 1% Neutridoma -SP (Boehringer Mannheim), and 0.4 µg / ml hydrocortisone (Upjohn, Rydalmere, NSW) (ECM-FCS), and human endometrial cells were cultured in DMEM-FCS.
Recombinant human (rh)TGF-β_1 was from R&D Systems, recombinant murine GM-CSF was provided by N. Nicola, The Walter and Eliza Hall Institute for Cancer Research, and recombinant human activin and inhibin were provided by J. Findlay, Prince Henry's Institute for Medical Research. Monoclonal antibodies (mAb) used for immunohistochemistry were anti-CD45 (TIB 122), anti-Mac-1 (CD11b, TIB 128), anti-MHC class II (Ia antigen, TIB 120; all from ATCC), F4/80 (15), and RB6-6C5 (16). Mouse anti-bovine TGF-$\beta_{1,2,3}$ mAb (which neutralizes all three mammalian TGF-β isoforms) was from Genzyme (Cambridge, MA) and chicken anti-bovine TGF-β1 mAb (neutralizes TGF-β1, <2% cross reactivity with TGF-β_2 and -β_3) was from R & D Systems.

Mice and Surgical Procedures. Adult (8-12 week) female mice of the [Balb/c X C57B1]F1, Balb/c or Balb/k strains, and adult male mice of the [CBA X C57B1]F1, CBA, or Balb/c strains were obtained from the University of Adelaide Central Animal House and maintained in a minimal security barrier facility on a 12 hour light / 12 hour dark cycle with food and water available *ad libitum*. Females were synchronised into estrus using the Whitten effect (17) and cycle stage was confirmed by analysis of vaginal smears. For natural mating, females were placed 2 per cage with individual males and the day of sighting of a vaginal plug was nominated as day 1 of pregnancy.

Male studs used for collection of accessory gland secretions were all of proven fertility and were rested for one week prior to use.

For intra-uterine injections, uterine horns of estrus females were exteriorised through a dorsal midline excision and injected with 0.2 - 40 ng rhTGF-β_1 in 50 ml of RPMI / 0.1% BSA, or vehicle only, prior to sacrifice of mice 16 hours later for assessment of luminal cytokine content or collection of uterine tissue for immunohistochemistry. Non-surgical administration of sperm / TGFβ_1 to the uterine lumen was achieved by passing a 3 French gauge Tom Cat™ catheter (Sherwood Medical, St. Louis, MO) into the uterine lumen (proximal to the point of bifurcation) of restrained females, after visualisation of the cervix with the aid of an auriscope (Heine, Germany), and manual dilation of the cervix with a fine wire. Each uterine catheter was loaded with 50 µl of sperm / TGFβ_1, which was delivered to the uterine cavity with the aid of a mouth pipette.

Vasectomised mice were prepared by bilateral ligation of the vas deferens through a transverse incision in the abdomen (Hogan *et al.*, 1986), and seminal vesiculectomised mice were prepared by removal of the seminal vesicles through a transverse incision in the abdomen following ligation and severing of the proximal tubule at the base of the gland. The body wall and skin were sutured and the mice were allowed to recover for at least two weeks prior to mating.

All surgical procedures were performed under anaesthesia using Avertin [1 mg / ml tribromoethyl alcohol in tertiary amyl alcohol (Sigma) diluted to 2.5% v / v in saline; 15 µl / g body weight injected i.p.].

Collection of Reproductive Tract Fluids. Seminal vesicle secretions were extruded from intact glands and solubilised in 6 M guanidine HCl (1:4 v / v), then desalted into DMEM using 5 ml Sephadex G-25 desalting columns (Pharmacia) before application to epithelial cell cultures. Prostate and coagulating gland secretions were extracted by homogenisation of intact glands in 0.5 ml of PBS / 1% BSA, followed by sedimenation of debris at 5000 g. Uterine luminal fluid was collected 16 h after mating or instillation of rhTGF-β1 into the uterus by flushing each horn with 500 µl of RPMI-FCS. Debris was sedimented at 2000 g and the supernatant stored at -80 °C prior to cytokine assay. In experiments where uterine TGF-β1 was measured, flushings of the right horn were made with 6 M guanidine HCl / 0.1% BSA, and desalted into PBS / 0.1% BSA prior to cytokine assay. For matings with intact and seminal vesicle deficient males the left horn

was flushed with DMEM to enable confirmation that adequate insemination had occurred (> 1 x 10^6 sperm per ml).

Chromatography. Approximately 1 ml of seminal vesicle fluid in 6 M guanidine HCl was applied to a Sephacryl S-400 column (40 cm x 16 mm; Pharmacia) equilibrated in 6 M guanidine HCl / 0.05 M Hepes pH 7.4. Fractions of 1 ml were collected, desalted into DMEM and assayed for GM-CSF-stimulating activity. Before addition to uterine culture or TGF-β assay half of each fraction was acid activated as previously described (18).

Murine uterine epithelial cell cultures. Uterine epithelial cells were prepared as previously described (19) and plated in 1 ml culture wells (Nunc) at 1-2 x 10^5 cells / ml in 500 µl of DMEM-FCS. After 4 h incubation at 37 °C in 5% CO2 to allow cell adherence, a further 500 µl of desalted seminal vesicle fluid in DMEM-FCS, cytokines in DMEM-FCS, or DMEM-FCS alone, were added. Culture supernatants were collected and replaced with fresh medium at 16 hours, then collected again 24 hours later, at which time adherent cells were quantified as previously described (19). All treatments were performed in duplicate or triplicate.

Human endometrial cultures. Human endometrial cell cultures were prepared under sterile conditions using a modification of the procedure described by Bentin-Ley (64). Briefly, stromal cells were embedded in a collagen matrix, covered by a thin layer of Matrigel (Collaborative Biomedical Products, Bedford, MA), which in turn was over-laid with uterine epithelial cells. Uterine epithelial cell supernatants were collected at 12 hrs (basal), replaced with 400 µl of medium containing either rTGFβ_1, semen, or fresh culture medium, and supernatants were collected 12 h later. The GM-CSF content of 24 h supernatants were normalised to the GM-CSF content of corresponding 12 h (basal) supernatants.

Human cervical keratinocytes. Human cervical keratinocytes were cultured using a modification of the technique described by Rheinwald and Green (65). Cervical biopsies were obtained from consenting women undergoing hysterectomy for non-malignant gynaecological indications. All women were pre-menopausal, but no distinction was made regarding stage of menstrual cycle at the time of surgery. The cervical biopsies were placed in ice-cold HBSS for transport to the laboratory, washed twice in antibiotic containing medium, and incubated overnight at 4 ^{0}C in DMEM containing 5 U dispase (Boehringer Mannheim). Large sheets of keratinocytes were mechanically stripped from the biopsy using sterile forceps after a subsequent 1 h

incubation at room temperature. Disaggregation into single cells was facilitated by incubation in DMEM / 0.25% trypsin / 0.05 % collagenase for 30 minutes at 37 °C, and repeated aspiration using a needle and syringe. Keratinocytes were cultured in ECM-FCS, at a density of 1-2 x 10^5 cells / ml, over monolayers of murine 3T3 fibroblasts rendered mitogenically inactive by exposure to 4% mitomycin C (Sigma). Keratinocytes were incubated for 5 -7 days to enable attachment and displacment of the 3T3 fibroblasts, when the media was replaced with fresh ECM-FCS. Supernatant was collected 12 h later (basal) and replaced with 500 µl of ECM-FCS containing 10 ng of rTGFβ_1, 10% semen or culture medium only (control), which in turn was collected 12 hrs later. The GM-CSF content of 24 h supernatants were normalised to the GM-CSF content of corresponding 12 h (basal) supernatants.

Cytokines and Cytokine Assays. GM-CSF was assayed using the GM-CSF dependant cell line FD5/12, essentially as previously described (19). Cell proliferation was determined by the addition of Alamar Blue (Alamar Biosciences) for the last 24 h of the assay or by pulsing with 1 µCi of [^{3}H]-thymidine per well for the last 6 h of the assay. The minimal detectable amount of GM-CSF was 1 U / ml (50 U / ml defined as that producing half maximal FD5/12 proliferation). TGF-β bioactivity was measured using Mv-1-Lu cells as previously described (71), except that cell numbers were quantified by the addition of Alamar Blue for the last 24 h of the assay. The minimal detectable amount of TGF-β in this assay was 15 pg / ml. Cytokine bioassays were standardised against recombinant cytokines and the specificity of the assays was confirmed by the use of cytokine specific neutralising antibodies. TGF-β1 immunoactivity was measured in a specific ELISA (R&D Systems) according to the manufacturers instructions.

Immunohistochemistry. Uterine tissue was embedded in OCT Tissue Tek (Miles Scientific) and frozen in isopropanol cooled by liquid N_2 , then stored at -80 °C until use. Six µm semi-serial sections were cut from uteri collected at 1400 h on the day of estrus or day 1 of pregnancy, or from mice injected with rhTGF-β1and fixed in 96% ethanol (4 °C / 10 min). For mAb staining, sections were incubated with mAbs (neat hybridoma supernatant containing 10% normal mouse serum [NMS]) and goat anti-rat-horseradish peroxidase (HRP; Dako, 1:20 in PBS containing 10% NMS) as detailed previously (19). To visualise HRP or endogenous peroxidase (to detect eosinophils), slides were incubated in diaminobenzidine (Sigma)(5 mg/ml in 0.05 M Tris-HCl pH 7.2) plus 0.02% hydrogen peroxide for 10 min at room temperature. After counterstaining in haematoxylin the sections were analysed using a video image

analysis package (Video Pro, Faulding Imaging, Adelaide) in which the area of positive staining in the endometrial stroma was expressed as a percentage of total cell staining.

Anti-sperm antibody ELISA: A solid phase ELISA technique modified from the protocol of Okada (66) was used to quantify the serum content of sperm-specific immunoglobulins in an isotype-specific manner. Antigen was prepared by disruption of freshly isolated CBA sperm (5×10^6 sperm / ml in PBS) using a Branson sonicator. 50 µl of sperm antigen suspension was added to polystyrene 96 well flat-bottomed ELISA plates (Maxisorb™, Nunc), and incubated overnight at 4 ^{0}C. Plates were blocked with PBS / 3% BSA for 1 h, and stored at -20 ^{0}C until use. Serum was diluted 1:4 in PBS, then serially diluted 1:2 to a final dilution of 1:128, before 2 h incubation in the thawed sperm antigen-coated plates. Bound immunoglobulin was detected with rabbit α mouse antibody (Mouse Typer™, BioRad; 1 hr), followed by biotinylated donkey α rabbit antibody (Amersham,UK; 1:2000 in PBS / 1% BSA; 1 hr) and streptavidin-HRP (Amersham; 1:4000 in PBS; 30 mins). HRP was visualised by the addition of tetra methylbenzidine (TMB, Sigma; 20 mins) following acidification of product with 1 M H_2SO_4. Quantification of each immunoglobulin isotype (IgG_1, IgG_{2a}, IgG_{2b}) was performed in duplicate, and all incubations were at room temperature. The antibody titre of each serum was determined by plotting A_{450} against titration.

Sperm antigen delayed type hypersensitivity (DTH) response: A footpad swelling assay (69) was employed to measure the DTH response against sperm antigens. Balb/c F1 mice were primed on two occasions separated by one month by intra-uterine inoculation with sperm antigens in the presence or absence of TGFβ , and 10 days later, footpad thickness was measured using a micrometer gauge (0.01 mm increments)(Mitutoyo, Tokyo, Japan) before and 24 h following injection into the hind footpad of 25 µl of sperm suspension (1×10^8 sperm / ml in HBSS). Antigen-specific swelling was calculated by subtracting the thickness of contralateral footpads injected with HBSS.

Human leukocyte chemotaxis assay: Leukocyte populations were obtained from human peripheral blood using Ficoll-Paque™ density gradient centrifugation, according to the method described by Boyum (68). Peripheral blood mononuclear cells (PBMC: lymphocytes and monocytes) were suspended in HBSS containing 10 % ECM-FCS at 5×10^5 cells / ml. The chemotaxis assay was a modification of a Boyden chamber protocol described by Bignold (69). Cervical keratinocyte culture supernatants (diluted 1:1 with HBSS / 10% ECM-FCS), HBSS / 10% ECM-FCS, or N-formyl-methionyl-

leucyl-phenylalanine (FMLP, Sigma) were added to the bottom half of chambers and were separated from PBMCs by 3 μm polycarbonate mounted adjacent to an 8 μm polycarbonate sparse-pore filter (Nuclepore). Following 45-60 mins incubation at 37oC, during which time PBMCs migrating through the 8 μm sparse-pore filter were trapped on the surface of the underlying 3 μm filter, cells were fixed by addition of 1 ml of 10% formalin and quantified by manual counting after staining with Mayer's haematoxylin. Mean cell numbers (± s.d.) of triplicate measurements were made for each test sample.

EXAMPLE 1.

Seminal TGFβ initiates the post mating inflammatory response in mice and humans

The cytokine GM-CSF, produced by the uterine epithelium following contact with seminal vesicle secretions, is thought to be pivotal to the generation of maternal tolerance since it is largely responsible for initiating the leukocyte influx into the female reproductive tract after mating and for increasing the antigen presenting capacity of these cells.

Seminal vesicle fluid was fractionated by size exclusion chromatography in order to identify GM-CSF-stimulating activity. Two fractions were identified; a high molecular weight (650 kDA) proteinacous moiety and a intermediate molecular weight, more heterogenous moiety eluting between 150-440 kDa (10,62). The latter moiety was identified as $TGF\beta_1$, on the basis of findings that it's GM-CSF stimulating activity was enhanced by acid activation, that $TGF\beta_1$ immunoactivity and bioactivity co-eluted in the same fraction, and that anti-$TGF\beta_1$ neutralising antibody could block the GM-CSF stimulating activity of this fraction (Figures 1,2). The molecular weight of the GM-CSF stimulating activity in seminal vesicle fluid (150-440 kDa) is consistent with that of the latent form of TGF-β_1, a complex of 230-290 kDa which comprises of the mature TGF-β dimer (25 kDa) non-covalently associated with a 75-80 kDa latency associated protein and a 130-190 kDa binding protein (23).

The TGF-β1 content of murine seminal vesicle secretions, like that of human seminal plasma (22), was found to be extraordinarily high and second only to that reported for platelet distillate (23). Furthermore the seminal vesicle gland secretions were identified as contributing in excess of 90% of total ejaculate $TGF\beta_1$ content, with the prostate and coagulating gland secretions containing only small amounts of $TGF\beta_1$. The addition of

rTGFβ$_1$ to uterine epithelial cells in culture and in vivo was confirmed to increase uterine epithelial GM-CSF output in a dose responsive manner (Figure 3).

The administration of rTGFβ1 to the uterine lumen of oestrus mice was observed to not only increase uterine GM-CSF production, but also initiate an influx and activation of inflammatory cells similar to that seen following mating (Table 1 and Figure 6). This result further supports the proposal that TGFβ can fully replicate the post-mating inflammatory response induced in the natural situation by seminal plasma.

In vitro experiments with human cervical keratinocytes and endometrial tissue indicated that both semen and rTGFβ$_1$ can elicit an increase in GM-CSF production from reproductive tract tissues in women (Figure 7). Furthermore, the content of leukocyte chemotactic activity in supernatants from keratinocyte cultures was enhanced by treatment with either semen or rTGFβ$_1$ (Figure 8), further supporting a principal role for seminal TGFβ in the post-mating inflammatory cascade in women (63).

Table 1. The effect of intra-uterine injection with TGF-β1 on endometrial leukocyte parameters.

treatment	n	CD45	F4/80	Mac-1	Ia	RB6-8C5	peroxidase
vehicle	5	15 (8-19)^a	15 (12-25)^a	9 (7-21)^a	20 (8-23)^a	11 (5-15)^a	4 (4-7)^a
rhTGF-β$_1$	4	28 (13-39)^{ab}	37 (30-48)^b	23 (18-42)^a	25 (15-35)^{ab}	15 (4-20)^a	15 (11-19)^b
mated	4	41 (30-60)^b	31 (21-49)^b	48 (46-56)^b	32 (26-57)^b	36 (15-41)^b	13 (10-20)^b

Tissues were collected 16 h after natural mating with intact males, or after administration of 20 ng rhTGF-β1 in 50 µl PBS / 1% BSA, or vehicle only, to the uterine luminal cavity of estrous mice. The reactivity of endometrial tissue with mAbs specific for all leukocytes (anti-LCA), macrophages (F4/80 and anti-Mac-1), neutrophils (anti-Mac-1 and RB6-8C5), and activated macrophages / dendritic cells (Ia), was determined by immunohistochemistry and video image analysis. Eosinophils were detected by staining for endogenous peroxidase activity (peroxidase). Reactivity with mAbs are expressed as the median (range) percent positivity. The number of mice in each experimental group = n. Data were compared by Kruskal-Wallis one way

ANOVA and Mann Whitney Rank Sum test. Data sets labelled with different lower case letters within columns denote statistical significance between treatment groups ($p < 0.01$).

EXAMPLE 2.

Seminal vesicle fluid modulates maternal reproductive performance and the maternal immune response to paternal antigens.

Previously, exposure to semen at mating was found to cause an intense but transient inflammatory response, and factors in seminal plasma derived from the seminal vesicle were implicated in this response. In studies in mice, the inventors have identified seminal vesicle fluid as a pivotal determinant in optimal embryo development and implantation. Furthermore, exposure to semen at mating has been shown to have an important role in inducing maternal tolerance prior to implantation, and factors present in seminal plasma have been identified as necessary for induction of this state, suggesting that the beneficial effect of seminal plasma on pregnancy outcome may at least in part be due to the immune deviating effects of this fluid.

To test the importance of exposure to seminal reside fluid for pregnancy success, Balb/c F1 females were mated with CBA males from which the seminal vesicles had been surgically removed (SV- studs). No implantation sites were present in the uterus on day 17 of pregnancy (n=12 females). This total infertility was not due to a lack of fertilisation, but rather was associated with implantation failure or early fetal resorption. This may reflect insufficient maternal tolerance of the semi-allogeneic embryos due to the lack or exposure to seminal reside TGFβ at mating.

Table II Effect of seminal plasma on embryonic development of mice.

	Intact	SV-
Number of females with embryos on day 3 (%)	8/8 (100%)	8/8 (100%)
# embryos @ day 3 (mean ±SD)	8.0±2.1	9.0±2.0
Number of females with implantation sites on day 17 (%)	10/10 (100%)	0/12 (0%)
# implants @ day 17 (mean±SD)	7.5±1.8	0

Balb/c F1 mice mated naturally with intact or seminal vesicle-deficient (SV-) CBA males were sacrificed at 1600h on day 3 to assess embryonic development, or on day 17 to determine number of implantation sites.

To investigate the importance of semen, particularly seminal vesicle fluid, on the induction of Th1 immune response to paternal MHC antigens, Balb/k (H-2^k) female mice were mated with intact Balb/k or congenic Balb/c (H-2^d) stud males, or Balb/c SV- studs. To achieve psuedopregnancy, the uteri of Balb/k females were ligated at the oviductal junction 2 weeks prior to mating. Immune responsiveness to MHC class I (H-2^d) antigen was assessed by measuring the growth of tumor cells injected on day 4 of pregnancy or psuedopregnancy. Tumor cells were rejected in most Balb/k females mated with Balb/k males, but grew in pregnant or psuedopregnant Balb/k females mated with Balb/c males. In contrast, tumors did not usually grow in Balb/k mice mated with SV- Balb/c males. These data demonstrate that exposure to semen is sufficient to induce specific tolerance to paternal MHC class I antigens, even in the absence of an ensuing pregnancy, and show that this tolerance is dependant on factors derived from the seminal vesicle (Table III).

Table III. Effect of pregnancy and psuedopregnancy on rejection of Balb/c JR-5 fibrosarcoma cells in Balb/k mice.

Female	Male	status at JR-5 injection	tumor growth at day 17 (%)	median tumor size#
Balb/c		virgin	11 / 11 (100)	++++
Balb/c	Balb/c	d4 pregnant	5 / 5 (100)	++++
Balb/k		virgin	0 / 10 (0)	-
Balb/k	Balb/c	d4 pregnant	13 / 14 (93)	+++
Balb/k	Balb/c (vas)	d4 psuedopregnant	5 / 7 (71)	++
Balb/k	Balb/c (SV-)	d4 pregnant	4 / 11 (36)	++
Balb/k (ut lig)	Balb/c	d4 psuedopregnant	9 / 9 (100)	+++
Balb/k	Balb/k	d4 pregnant	5 / 15 (33)	+
Balb/k	C57Blk x CBA	d4 pregnant	4 / 8 (50)	+
Balb/k (ut lig)	C57Blk x CBA	d4 psuedopregnant	4 / 8 (50)	+

Balb/c (H-2d) or Balb/k (H-2k) female mice were mated with Balb/c or C57Blk x CBA F1 (H-2b/k) studs. In some groups the uteri of Balb/k females were ligated at the oviductal junction 2 weeks prior to mating (ut lig). Other groups of intact Balb/k mice were mated with vasectomised Balb/c males (vas) or Balb/c males from which the seminal vesicles were removed at least 2 weeks prior to mating (SV-). The day of finding a vaginal plug was designated day 1 of pregnancy or psuedopregnancy. Balb/c tumor cells (JR-5 fibrosarcoma cells, 10^5) were injected s.c. on day 4, and tumor growth (diameter, in two dimensions) was measured on day 17 of pregnancy or psuedopregnancy (++++ = > 8 mm; +++ = > 5 mm; + = 1-3 mm).

EXAMPLE 3.

Seminal TGFβ is an immune deviating agent.

To assess the effect of TGFβ on induction of Th1 and Th2 immune responses against CBA sperm antigens, Balb/c F1 female mice were immunised by intra-uterine infusion with CBA sperm, in the presence or absence of rTGFβ, on two occasions separated by 4 weeks. Development of Th1 anti-sperm immunity was assessed two weeks later by

measuring the DTH response to a subcutaneous sperm antigen challenge, and by measuring serum content of anti-sperm reactive immunoglobulin of the IgG_{2b} subclass. Whereas sperm administered alone or in the presence of Freunds Complete Adjuvant elicited a strong DTH response and a moderate IgG2b antibody response, immunisation in the presence of TGFβ substantially diminished both of these parameters, and was comparable to the response elicited by natural mating (Figure 8). In contrast, synthesis of sperm-reactive immunoglobulin of the IgG1 isotype (indicating induction of a Th2 response) occurred to a similar extent in all treatment groups, regardless of the presence of TGFβ in the immunising inoculum.

In another experiment, the effect of TGFβ on the induction of 'tolerance' to paternal MHC antigens associated with sperm was investigated. Balb/k (H-2k) female mice that were given intra-uterine infusions of sperm from Balb/c (H-2d) males together with $rTGF\beta_1$ were not able to reject paternal MHC antigen-bearing tumour cells injected 4 days later, whereas tumours were rejected in naïve mice or mice given sperm alone (Table IV). Tumour rejection was also compromised in mice that administered TGFβ without sperm antigen, although tumours in this treatment group were not as large as those which grew in mice that received both antigen and TGFβ.

Both of these experiments show that delivery of paternal antigens in combination with TGFβ to the female reproductive tract can generate systemic paternal antigen-specific tolerance, specifically by inhibiting the Th1 compartment of the immune response. This immune deviating effect is dependent on the administration of TGFβ since antigen given alone elicits Th1 immunity as opposed to tolerance. TGFβ given in the absence of antigen may confer a state of partial, non-antigen specific tolerance.

Table IV: The effect of intra-uterine immunisation with Balb/c sperm and TGFβ on rejection of Balb/c JR-5 fibrosarcoma cells in virgin Balb/k mice.

Treatment	tumor growth at day 17 (%)	median tumor size#
5×10^6 Balb/c sperm	3 / 8 (38)	+
10 ng TGFβ	5 / 7 (71)	+++
5×10^6 Balb/c sperm + 10 ng TGFβ	6 / 9 (67)	++++
Control (PBS)	0 / 6 (0)	-

Balb/k female mice were uterine ligated, and after two weeks rest were synchronised into estrous by administration of GnRH agonist. At 0900 h – 1200 h on the day of estrous, mice were anaesthetised and given intra-uterine injections of 5 x 10^6 Balb/c sperm and/or 10 ng TGFβ in 100 ul of PBS (50 ul administered per horn). Balb/c tumor cells (JR-5 fibrosarcoma cells, 10^5) were injected s.c. 72 h after surgery, and tumor growth (diameter, in two dimensions) was measured 13 days later (++++ = > 8 mm; +++ = > 5 mm; + = 1-3 mm).

EXAMPLE 4.

Paternal antigen-specific immune deviation improves reproductive performance

The experiments described above show that seminal vesicle secretions can elicit Th1 hypo-responsiveness which manifests as 'tolerance' in the maternal immune response specific for seminal antigens, including but not likely to be limited to paternal MHC antigens, deposited in the female reproductive tract at mating. The data suggest that diminished reproductive outcome ensues when a pregnancy has been initiated in the absence of exposure to seminal plasma, perhaps because of inadequate induction of maternal 'tolerance' to conceptus antigens. An experiment was therefore performed to test the hypothesis that a prior state of TGFβ-mediated 'tolerance' to antigens in paternal semen can benefit reproductive performance. This experiment consisted of immunisation by intra-uterine infusion of Balb/c F1 females with CBA sperm, with or without $rTGF\beta_1$, two weeks before mating with intact CBA male studs. Immunisation with sperm plus $TGF\beta_1$ resulted in an increase in mean fetal and placental weight (Table V), despite a small decline in litter size which was evident in all females immunised with sperm regardless of the presence of TGFβ. This increase was still apparent after adjustment for different fetal numbers per uterine horn, thereby discounting an effect of litter size (Figure 9).

Induction of Th1 hypo-responsiveness against paternal antigens has been reported to result in an improved pregnancy outcome in women previously experiencing recurrent miscarriage (102). While no data exist on the ability of paternal antigen / TGFβ immunisation to initiate Th1 hypo-responsiveness against paternal antigens, or to deviate previously existing Th1 immune responses in women, nor on the ability of TGFβ to improve reproductive outcome, this is likely to be the case. The inventors have been the first to conduct a large randomised, controlled trial investigating the effect of semen exposure on IVF treatment outcome. This trial has confirmed that women exposed to semen (containing paternal antigen and natural TGFβ) around the time of

thawed embryo transfer have a reduced risk of early embryonic loss compared to those instructed to abstain (Table VI). This improvement in reproductive outcome is likely to be mediated by maternal immune tolerance towards paternal antigens initiated by TGFβ and seminal antigens at the time of intercourse.

Table V. Effect of prior immunisation with sperm and TGFβ on reproductive outcome in mice

	Control	sperm + $TGF\beta_1$	sperm
number	139	144	103
litter size (total)	11.4 ± 1.0^{a}	10.4 ± 1.2^{b}	10.3 ± 0.9^{b}
litter size (viable)	11.25 ± 1.3^{a}	10.1 ± 1.5^{b}	10.1 ± 0.9^{b}
# resorptions	0.167 ± 0.58^{a}	0.21 ± 0.58^{a}	0.20 ± 0.42^{a}
fetal weight (mg)	645.2 ± 61.2^{a}	677.6 ± 56.6^{b}	646.1 ± 49.9^{a}
placental weight (mg)	97.7 ± 12.1^{a}	105.2 ± 12.4^{b}	101.8 ± 9.8^{b}
fetal:placental weight ratio	6.69 ± 0.9^{a}	6.5 ± 0.8^{ab}	6.36 ± 0.8^{b}

Balb/cF1 female mice were immunised by intra-uterine infusion with CBA sperm in the presence or absence of 10ng $rTGF\beta_1$, and were mated naturally with CBA males 2 weeks later. Females were sacrificed on day 17 of pregnancy and the number of total, viable and resorbing implantation sites, as well as fetal and placental weights of viable conceptuses, were determined. Values are mean±SD. Comparisons between groups were by Kruskal Wallis one-way ANOVA followed by Mann Whitney rank sum test ($p < 0.05$).

Table VI. Effect of semen exposure around the time of thawed embryo transfer on early pregnancy outcome.

	Intercourse	abstain	significance
transfer cycles	59	56	NS
embryos transferred	106	107	NS
implantations (%)	11/106 (10.3)	11/107 (10.2)	NS
viable conceptus at 6 weeks (%)	10/106 (9.4)	7/107 (6.5)	NS
transfer cycles with biochemical pregnancy	9/59* (15.3)	7/56 (12.5)	NS
biochemical pregnancy loss	0 (0)	2/11 (8.2)	NS
clinical miscarriage	1/11 (9)	2/11 (18.2)	NS
total pregnancy wastage	1/11 (9)	4/11 (36.4)	0.043

Pregnancy outcome following thawed embryo transfer. Patient characteristics were not significantly different between the two groups. An biochemical pregnancy was defined as one serum βHCG exceeding 25 IU and a clinical pregnancy as a conceptus/ fetal pole seen at ultrasound at 6 weeks gestation. Statistical analysis was performed using the Chi square calculation. NS = not significant. * = one twin pregnancy.

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CLAIMS

1. A method of treating an infertility condition in a human or mammal by exposure of the prospective mother to TGFβ or an effective derivative or analog thereof before attempted conception to elicit a transient hyporesponsive immune reaction to one or more antigen of a prospective father to thereby alleviate symptoms of the infertility condition.

2. A method of treating an infertility condition as in claim 1 by exposure of a prospective mother to said one or more antigens of a prospective father and to TGFβ or an effective derivative or analog thereof before attempted conception to elicit a transient hyporesponsive immune reaction to said one or more antigen to thereby alleviate symptoms of the infertility condition.

3. A method of treating an infertility condition as in claim 2 wherein a mucosal surface of the prospective mother is exposed to the one or more antigens.

4. A method of treating an infertility condition as in claim 3 wherein the mucosal surface is selected from the group comprising an oral mucosal surface, a respiratory mucosal surface, a gastrointestinal mucosal surface or a genital mucosal surface.

5. A method of treating an infertility condition as in claim 3 wherein the mucosal surface is a genital mucosal surface.

6. A method of treating an infertility condition as in claim 3 wherein the one or more antigens and TGFβ or derivative or analog thereof is injected for systemic contact.

7. A method of treating an infertility condition as in claim 3 wherein the TGFβ or derivative or analog thereof and the one or more antigens are adminstered at one site.

8. A method of treating an infertility condition as in claim 3 wherein the TGFβ or derivative or analog thereof and the one or more antigens are each adminstered at a first site and a different site respectively.

9. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof and the one or more antigen are adminstered temporarily spaced apart.

10. A method of treating an infertility condition as in claim 9 wherein the one ore more antigens are adminstered subsequent to administration of the TGFβ or derivative or analog thereof.

11. A method of treating an infertility condition as in claim 9 wherein the one or more antigens are administered first followed by administration of TGFβ or derivative or analog thereof.

12. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are chosen as a result of being particularly antigenic and prominent either on the sperm, or on the conceptus.

13. A method of treating an infertility condition as in claim 2 wherien the one or more antigens are present on cells taken from the prospective father that contain MHC antigens.

14. A method of treating an infertility condition as in claim 13 wherein the antigen is an MHC I antigen of the prospective father.

15. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are administered on leukocytes of the prospective father.

16. A method of treating an infertility condition as in claim 2 wherin the one or more antigens are adminsurated on sperm cells of the prospective father.

17. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are adminsurated in the seminal plasma of the prospective father.

18. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are presented in purified or semi-purified form.

19. A method of treating an infertility condition as in claim 18 wherein the purified or semi purified one or more antigens are presented on inert or adjuvant carriers.

20. A method of treating an infertility condition as in claim 2 wherein humans are being treated, and the exposure of TGFβ is to a mucosal surface and the level of TGFβ is greater than 50 ng/ml with a total dose of 150ng/ml.

21. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is exposed to a concentration of TGFβ of between 100 and 400ng/ml with a total dose of between 100 to 2000ng.

22. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereofis supplied in a slow release form.

23. A method of treating an infertility condition as in claim 2 wherein the exposure of the one or more antigens is to the prospective mother's genital tract in the form of the prospective father's ejaculate, and the level of exposure is determined by the cell count and antigenic density on the surface of such cells.

24. A method of treating an infertility condition as in claim 3 wherein humans are being treated and the one or more antigens are present on leukocytes, whereby between 10^7 and 10^9 leukocytes are adminstered to a mucosal surface.

25. A method of treating an infertility condition as in claim 2 wherein the TGFβ is selected from the group of $TGF\beta_1$, $TGF\beta_2$ and $TGF\beta_3$.

26. A method of treating an infertility condition as in claim 2 wherein the TGFβ is $TGF\beta_1$.

27. A method of treating an infertility condition as in claim 2 wherein the TGFβ is modified.

28. A method of treating an infertility condition as in claim 27 wherein the modification is selected from the group comprising substitution, deletion or addition mutants, peptide fragments of TGFβ or derivative or analog thereof, and peptide fragments of TGFβ or derivative or analog thereof which have been incorporated into another protein.

29. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof is a member of the TGFβ superfamily.

30. A method of treating an infertility condition as in claim 29 wherein the member of the TGFβ superfamily is activin.

31. A method of treating an infertility condition as in claim 2 wherein TGFβ is administered in its active form.

32. A method of treating an infertility condition as in claim 2 wherein TGFβ is administered in precursor form.

33. A method of treating an infertility condition as in claim 2 wherein the prospective mother is incapable of converting sufficient of the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of active TGFβ.

34. A method of treating an infertility condition as in claim 2 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of a compound capable of activating TGFβ.

35. A method of treating an infertility condition as in claim 2 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of plasmin, so as to increase the level of active TGFβ.

36. A method of treating an infertility condition as in claim 2 wherein TGFβ is administered in an unpurified form using a biological source rich in TGFβ.

37. A method of treating an infertility condition as in claim 36 wherein the TGFβ is administered in the form of platelets.

38. A method of treating an infertility condition as in claim 3 wherein humans are being treated and the exposure to TGFβ and male antigen is a multiple exposure.

39. A method of treating an infertility condition as in claim 38 wherein the multiple exposure is preferably performed over a period spanning at least three months prior to attempted conception.

40. A method of treating an infertility condition as in claim 2 wherein humans are being treated and exposure is at least one week before conception is attempted.

41. A method of treating an infertility condition as in claim 2 wherein the exposure is before attempted conception.

42. A method of treating an infertility condition as in claim 2 wherein administration of TGFβ or derivative or analog thereof and the one ore more antigen occurs at least once after the prospective date of conception.

43. A method of treating an infertility condition as in claim 42 wherein the exposure continues over a period of the first 12 weeks of pregnancy.

44. A method of treating an infertility condition as in claim 2 first including the step of diagnosing or testing whether the male has adequate levels of TGFβ or the female has the capacity to activate TGFβ, or alternatively whether anti-sperm antibodies exist.

45. A method of treating an infertility condition as in claim 2 used in conjunction with IVF treatment, whereby the transient hyporeactive immune response is elicited before transfer of the conceptus or gametes is attempted.

46. A method of diagnosing an infertility condition in males by testing the level of TGFβ in seminal fluid.

47. A method of diagnosing an infertility condition in a female by testing for the capacity of the female to convert the inactive form of TGFβ to the active form.

48. A composition for use in treating an infertility condition, comprising TGFβ or derivative or analog thereof and one or more paternal antigens, and a pharmaceutically acceptable carrier, suitable for administration to a mucosal surface.

49. A composition for use in treating an infertility condition as in claim 48 wherein the composition comprises a vaginal gel.

AMENDED CLAIMS

[received by the International Bureau on 31 July 1998 (31.07.98); original claims 1-49 replaced by amended claims 1-48 (5 pages)]

1. A method of treating an infertility condition in a human or mammal by exposure of a prospective mother to one or more antigens of a prospective father and to substantially purified TGFβ or an effective derivative or analog thereof before attempted conception to elicit an immune reaction leading to tolerance to said one or more antigens to thereby alleviate symptoms of the infertility condition.

2. A method of treating an infertility condition as in claim 1 wherein a mucosal surface of the prospective mother is exposed to the one or more antigens.

3. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is selected from the group comprising an oral mucosal surface, a respiratory mucosal surface, a gastrointestinal mucosal surface or a genital mucosal surface.

4. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is a genital mucosal surface.

5. A method of treating an infertility condition as in claim 2 wherein the one or more antigens and TGFβ or derivative or analog thereof is injected for systemic contact.

6. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof and the one or more antigens are adminstered at one site.

7. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof and the one or more antigens are each adminstered at a first site and a different site respectively.

8. A method of treating an infertility condition as in claim 1 wherein the TGFβ or derivative or analog thereof and the one or more antigen are adminstered temporarily spaced apart.

9. A method of treating an infertility condition as in claim 8 wherein the one or more antigens are administered subsequent to administration of the TGFβ or derivative or analog thereof.

10. A method of treating an infertility condition as in claim 8 wherein the one or more antigens are adminstered first followed by administration of TGFβ or derivative or analog thereof.

11. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are chosen as a result of being particularly antigenic and prominent either on the sperm, or on the conceptus.

12. A method of treating an infertility condition as in claim 1 wherien the one or more antigens are present on cells taken from the prospective father that contain MHC antigens.

13. A method of treating an infertility condition as in claim 12 wherein the antigen is an MHC I antigen of the prospective father.

14. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are administered on leukocytes of the prospective father.

15. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are adminstrated on sperm cells of the prospective father.

16. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are adminstrated in the seminal plasma of the prospective father.

17. A method of treating an infertility condition as in claim 1 wherein the one or more antigens are presented in purified or semi-purified form.

18. A method of treating an infertility condition as in claim 17 wherein the purified or semi purified one or more antigens are presented on inert or adjuvant carriers.

19. A method of treating an infertility condition as in claim 2 wherein humans are being treated, and the exposure of TGFβ is to a mucosal surface and the level of TGFβ is greater than 50 ng/ml with a total dose of 150ng/ml

20. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is exposed to a concentration of TGFβ of between 100 and 400ng/ml with a total dose of between 100 to 2000ng.

21. A method of treating an infertility condition as in claim 1 wherein the TGFβ or derivative or analog thereofis supplied in a slow release form.

22. A method of treating an infertility condition as in claim 1 wherein the exposure of the one or more antigens is to the prospective mother's genital tract in the form of the prospective father's ejaculate, and the level of exposure is determined by the cell count and antigenic density on the surface of such cells.

23. A method of treating an infertility condition as in claim 2 wherein humans are being treated and the one or more antigens are present on leukocytes, whereby between 10^7 and 10^9 leukocytes are adminstered to a mucosal surface.

24. A method of treating an infertility condition as in claim 1 wherein the TGFβ is selected from the group of $TGF\beta_1$, $TGF\beta_2$ and $TGF\beta_3$.

25. A method of treating an infertility condition as in claim 1 wherein the TGFβ is $TGF\beta_1$.

26. A method of treating an infertility condition as in claim 1 wherein the TGFβ is modified.

27. A method of treating an infertility condition as in claim 26 wherein the modification is selected from the group comprising substitution, deletion or addition mutants, peptide fragments of TGFβ or derivative or analog thereof, and peptide fragments of TGFβ or derivative or analog thereof which have been incorporated into another protein.

28. A method of treating an infertility condition as in claim 1 wherein the TGFβ or derivative or analog thereof is a member of the TGFβ superfamily.

29. A method of treating an infertility condition as in claim 28 wherein the member of the TGFβ superfamily is activin.

30. A method of treating an infertility condition as in claim 1 wherein TGFβ is administered in its active form.

31. A method of treating an infertility condition as in claim 1 wherein TGFβ is administered in precursor form.

32. A method of treating an infertility condition as in claim 1 wherein the prospective mother is incapable of converting sufficient of the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of active TGFβ.

33. A method of treating an infertility condition as in claim 1 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of a compound capable of activating TGFβ.

34. A method of treating an infertility condition as in claim 1 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of plasmin, so as to increase the level of active TGFβ.

35. A method of treating an infertility condition as in claim 1 wherein TGFβ is administered in an unpurified form using a biological source rich in TGFβ.

36. A method of treating an infertility condition as in claim 35 wherein the TGFβ is administered in the form of platelets.

37. A method of treating an infertility condition as in claim 2 wherein humans are being treated and the exposure to TGFβ and male antigen is a multiple exposure.

38. A method of treating an infertility condition as in claim 37 wherein the multiple exposure is preferably performed over a period spanning at least three months prior to attempted conception.

39. A method of treating an infertility condition as in claim 1 wherein humans are being treated and exposure is at least one week before conception is attempted.

40. A method of treating an infertility condition as in claim 1 wherein the exposure is before attempted conception

41. A method of treating an infertility condition as in claim 1 wherein administration of TGFβ or derivative or analog thereof and the one ore more antigen occurs at least once after the prospective date of conception

42. A method of treating an infertility condition as in claim 41 wherein the exposure continues over a period of the first 12 weeks of pregnancy.

43. A method of treating an infertility condition as in claim 1 first including the step of diagnosing or testing whether the male has adequate levels of TGFβ or the female has the capacity to activate TGFβ, or alternatively whether anti-sperm antibodies exist.

44. A method of treating an infertility condition as in claim 1 used in conjunction with IVF treatment, whereby the transient hyporeactive immune response is elicited before transfer of the conceptus or gametes is attempted.

45. A method of diagnosing an infertility condition in males by testing the level of TGFβ in seminal fluid.

46. A method of diagnosing an infertility condition in a female by testing for the capacity of the female to convert the inactive form of TGFβ to the active form.

47. A composition for use in treating an infertility condition, comprising TGFβ or derivative or analog thereof and one or more paternal antigens, and a pharmaceutically acceptable carrier, suitable for administration to a mucosal surface.

48. A composition for use in treating an infertility condition as in claim 47 wherein the composition comprises a vaginal gel.

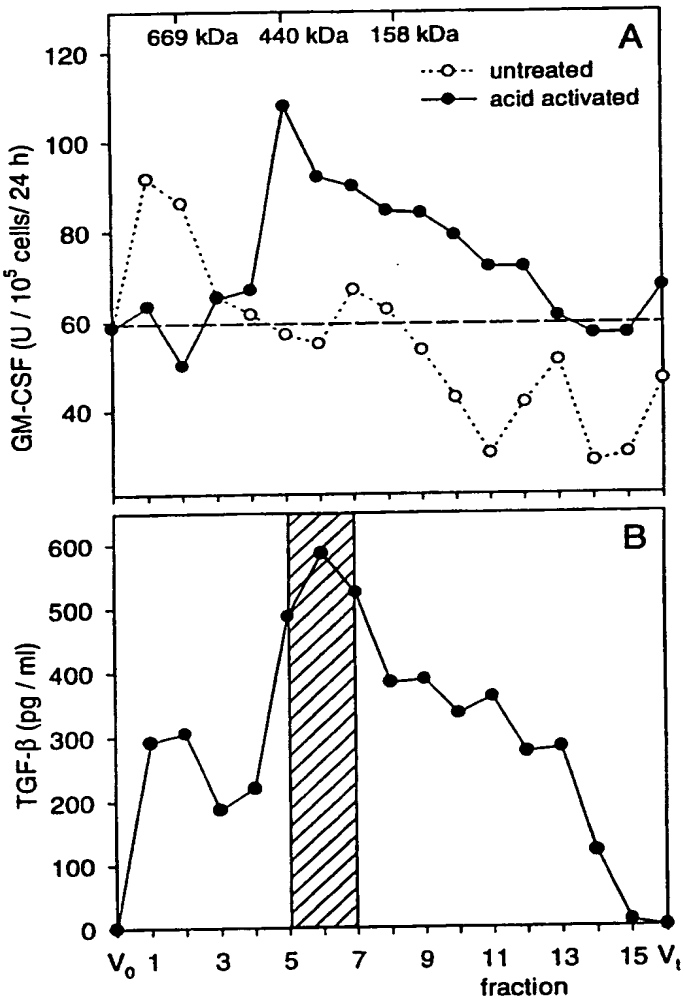
STATEMENT UNDER ARTICLE 19

1. Claim 1 has been cancelled.

2. Claim 2 has been amended and renumbered as claim 1.

3. Claims 3 to 49 inclusive have been renumbered 2 to 48 inclusive and the appendences have been amended accordingly.

For your convenience retyped pages 32 to 36 containing these amendments is fowarded together with a redlined copy of the Claims showing where the amendments have been made.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440 kDa
158 kDa
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untreated
acid activated
GM-CSF (U / 10^5 cells/ 24 h)
120
100
80
60
40
B
TGF-β (pg / ml)
600
500
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13
15
Vt
fraction

Figure 1

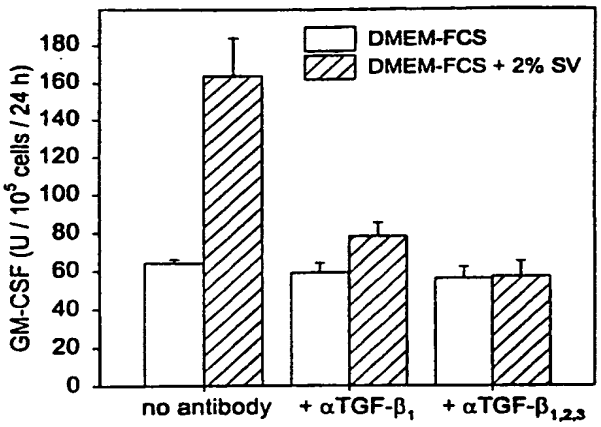
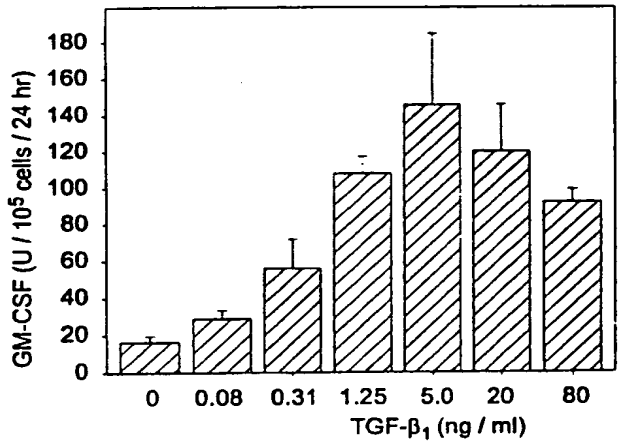


Figure 2



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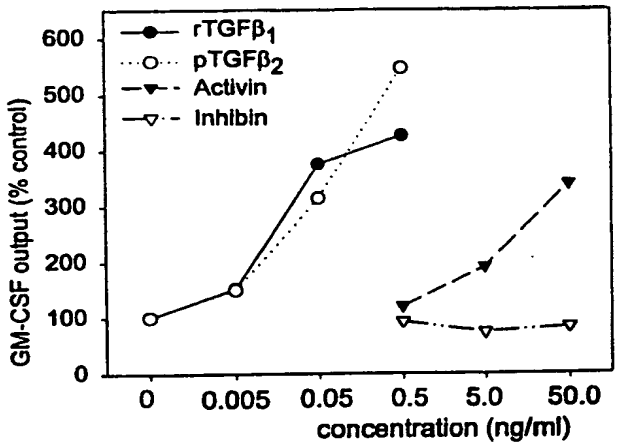
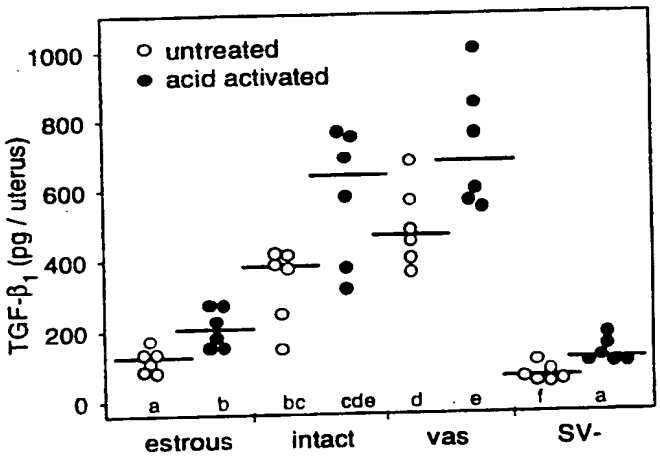


Figure 4



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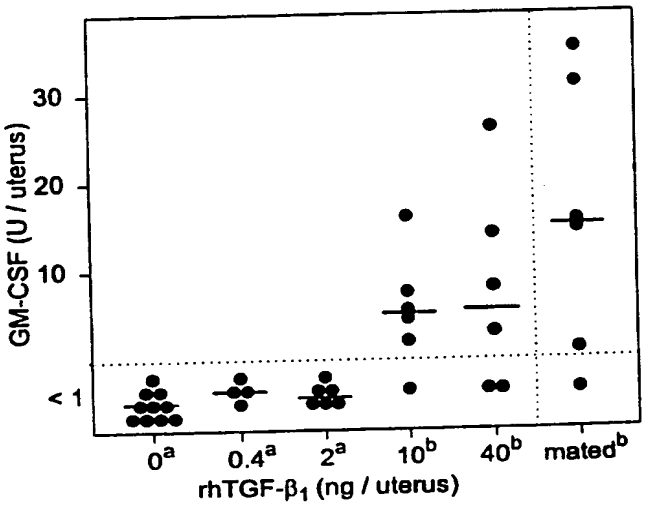
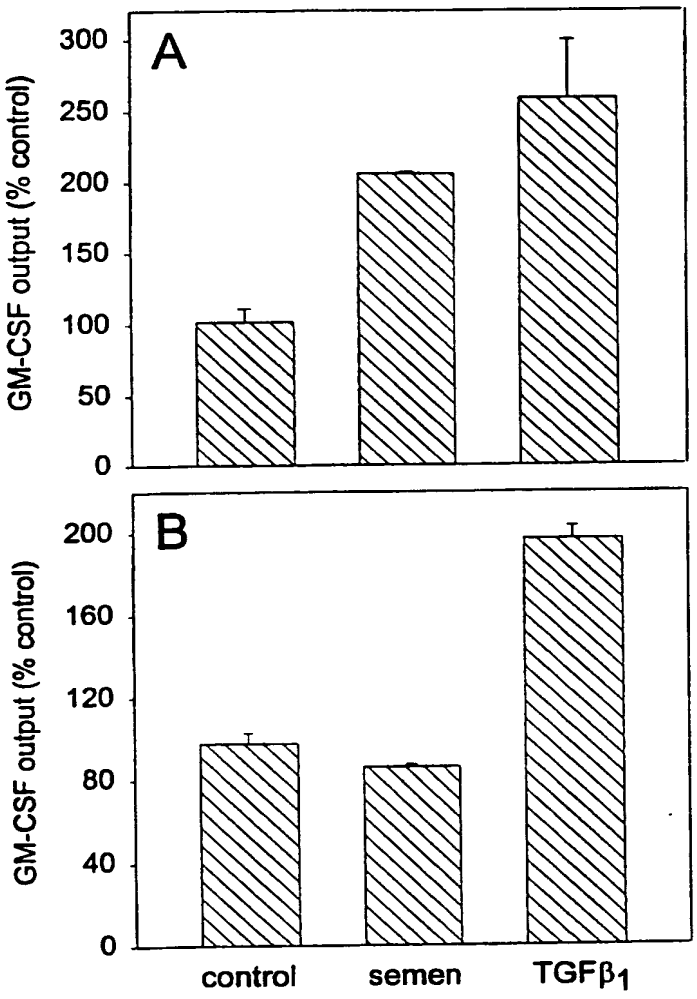


Figure 6


A
B
GM-CSF output (% control)
GM-CSF output (% control)
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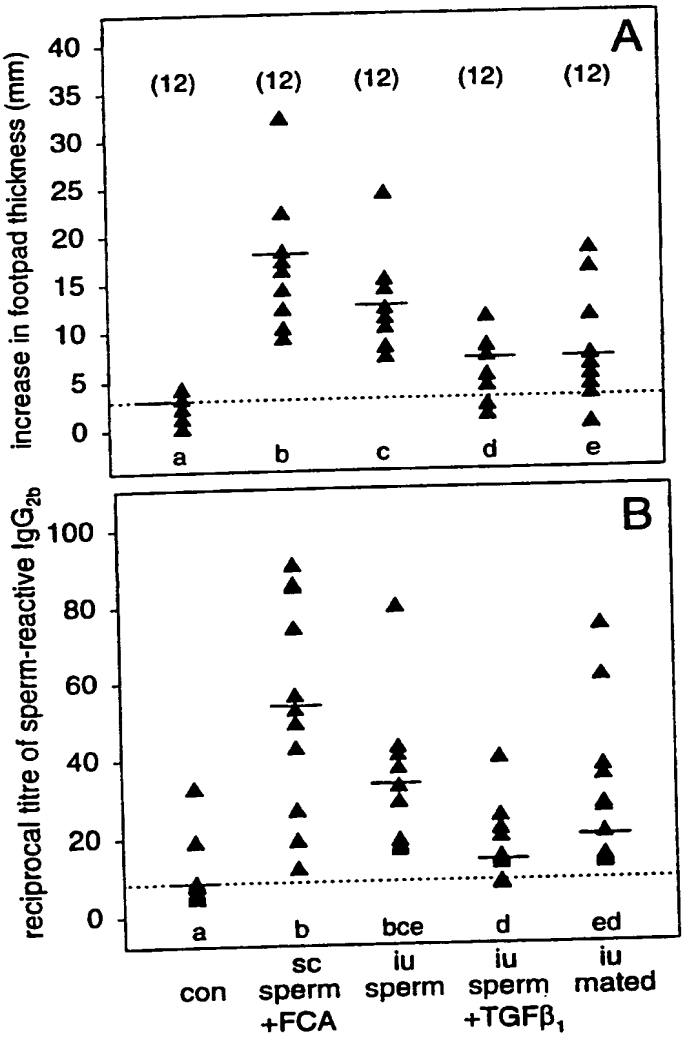
100
50
0
200
160
120
80
40
0
control
semen
TGFβ1

Figure 7


A
B
increase in footpad thickness (mm)
reciprocal titre of sperm-reactive IgG2b
(12)
(12)
(12)
(12)
(12)
a
b
c
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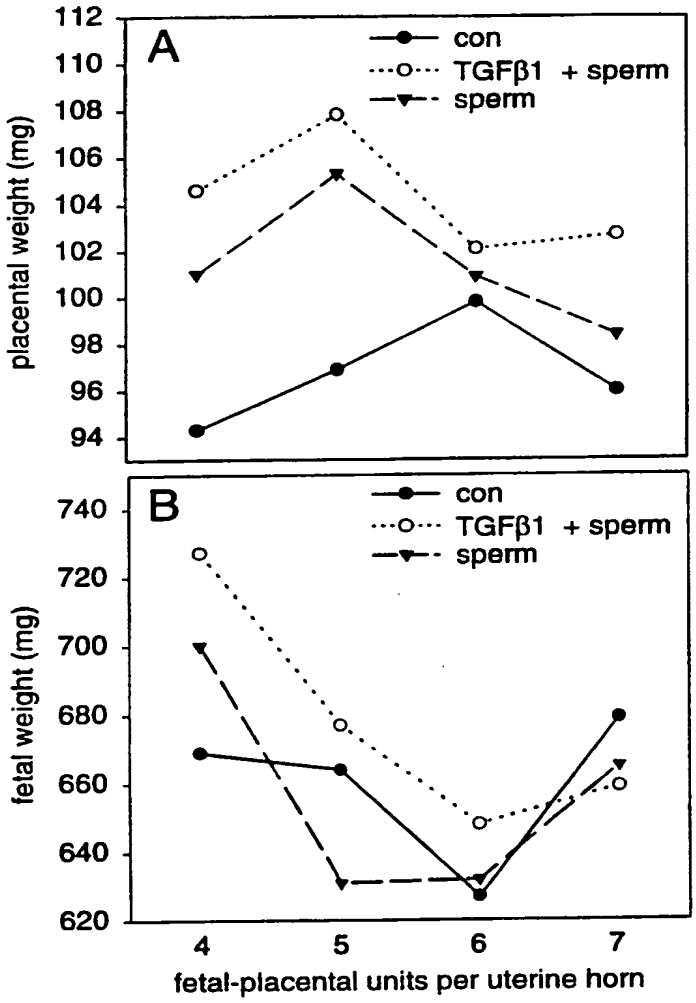
a
b
bce
d
ed
con
sc sperm +FCA
iu sperm
iu sperm +TGFβ1
iu mated

Figure 8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TGFβ1 + sperm
sperm
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108
106
104
102
100
98
96
94
B
con
TGFβ1 + sperm
sperm
fetal weight (mg)
740
720
700
680
660
640
620
4
5
6
7
fetal-placental units per uterine horn

Figure 9

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00149

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 38/18, 39/00, G01N 33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
AU:See search terms below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT, MEDLINE. SEARCH TERMS: INFERTIL, CONCEPTION, PREGNAN, FERTIL, ABORTION, MISCARRIAGE, PATERNAL, SPERM, MALE, TGF, TRANSFORMING GROWTH FACTOR, ACTIVIN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Journal of Clinical Immunology, Sept (1997) Vol 17(5) U.Gafter et. al. "Suppressed cell-mediated imunity and monocyte and natural killer cell activity following allogenic immunization". Pages 408-419, especially page 418, last paragraph.	1-49

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
26 MAY 1998

Date of mailing of the international search report
-2 JUN 1998

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00149

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Fertility and Sterility, vol 66(2) 1996, T.M.chu et. al. "Localisation of seminal plasma transforming growth factor - beta 1 on human spermatozoa: an immunocytochemical study." Pages 327 to 330, especially page 327 last paragraph, page 330 last paragraph.	1-49
X	American Journal of Reproductive Immunology, vol 33(4), 1995 M.Nocera and T.M.Chu "Characterisation of Latent Transforming Growth Factor - beta from human seminal plasma" pages 282 to 291. Whole document	1-49
X	WO 91/10445 (GENENTECH, INC) 25 July 1991 (For example, claim 1)	1
X	US 5395825 (YALE UNIVERSITY and TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 7 March 1995 (For example, column 3 line 66 to column 4 line 3, claims 3 and 4) (For example, column 3 lines 59 to 65, column 6 line 9 to 12 and line 23 to 26)	1 47
X, Y	US 5166190 (GENETECH, INC) 24 November 1992	46
Y	WO 95/04931 (SURFACE ACTIVE LIMITED) 16 February 1995	46, 47

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 98/00149

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9110445	AU	71734/91	EP	509040	WO	91/10445
US	5395825	AU	63998/94	CA	2156613	EP	688365
		WO	94/20637	US	5693479		
US	5166190	AU	71504/91	EP	510073	WO	91/10444
WO	95/04931	AU	73476/94	GB	9316369		

END OF ANNEX

A.P.T. PATENT AND TRADE MARK ATTORNEYS

Patents Trade Marks Registered Designs Copyright Licensing Searching

Your Ref:
Our Ref: 1103PCT:PJW:JML

31st July 1997

URGENT

International Bureau of WIPO
34 chemin des Colombettes
1211 Geneva 20
SWITZERLAND

Due Date - 4th August 1997

Facsimile - ~~10~~ 12 sheets

Dear Sirs

Patent Cooperation Treaty Application No PCT/AU98/00149
Treatment and Diagnosis of an Infertility Condition
LUMINIS PTY LTD

In response to the international search report dated 2nd June 1998 please find enclosed a Statement of Amendments together with retyped pages containing the amended claims..

Please confirm safe receipt and filing of these amendments, in due course.

Yours faithfully
A.P.T. Patent and Trade Mark Attorneys

Paul Wyk

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31st July 1998

Patent Cooperation Treaty Application No. PCT/AU98/00149
Treatment and Diagnosis of an Infertility Condition
LUMINIS PTY LTD

STATEMENT OF AMENDMENTS

1. Claim 1 has been cancelled.

2. Claim 2 has been amended and renumbered as claim 1.

3. Claims 3 to 49 inclusive have been renumbered 2 to 48 inclusive and the appendences have been amended accordingly.

For your convenience retyped pages 32 to 36 containing these amendments is fowarded together with a redlined copy of the Claims showing where the amendments have been made.

CLAIMS

~~1. A method of treating an infertility condition in a human or mammal by exposure of the prospective mother to TGFβ or an effective derivative or analog thereof before attempted conception to elicit a transient hyporesponsive immune reaction to one or more antigen of a prospective father to thereby alleviate symptoms of the infertility condition.~~

~~2~~ 1. A method of treating an infertility condition ~~as in claim 1~~ [in a human or mammal] by exposure of a prospective mother to ~~said~~ one or more antigens of a prospective father and to substantially purified TGFβ or an effective derivative or analog thereof before attempted conception to elicit ~~a transient hyporesponsive~~ an immune reaction leading to tolerance to said one or more antigens to thereby alleviate symptoms of the infertility condition.

~~3~~ 2. A method of treating an infertility condition as in claim ~~2~~ 1 wherein a mucosal surface of the prospective mother is exposed to the one or more antigens.

~~4~~ 3. A method of treating an infertility condition as in claim ~~3~~ 2 wherein the mucosal surface is selected from the group comprising an oral mucosal surface, a respiratory mucosal surface, a gastrointestinal mucosal surface or a genital mucosal surface.

~~5~~ 4. A method of treating an infertility condition as in claim ~~3~~ 2 wherein the mucosal surface is a genital mucosal surface.

~~6~~ 5. A method of treating an infertility condition as in claim ~~3~~ 2 wherein the one or more antigens and TGFβ or derivative or analog thereof is injected for systemic contact.

~~7~~ 6. A method of treating an infertility condition as in claim ~~3~~ 2 wherein the TGFβ or derivative or analog thereof and the one or more antigens are adminstered at one site.

~~8~~ 7. A method of treating an infertility condition as in claim ~~3~~ 2 wherein the TGFβ or derivative or analog thereof and the one or more antigens are each adminstered at a first site and a different site respectively.

~~9~~ 8. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the TGFβ or derivative or analog thereof and the one or more antigen are adminstered temporarily spaced apart.

~~10~~ 9. A method of treating an infertility condition as in claim ~~9~~ 8 wherein the one or more antigens are administered subsequent to administration of the TGFβ or derivative or analog thereof.

~~11~~ 10. A method of treating an infertility condition as in claim ~~9~~ 8 wherein the one or more antigens are adminstered first followed by administration of TGFβ or derivative or analog thereof.

~~12~~ 11. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the one or more antigens are chosen as a result of being particularly antigenic and prominent either on the sperm, or on the conceptus.

~~13~~ 12. A method of treating an infertility condition as in claim ~~2~~ 1 wherien the one or more antigens are present on cells taken from the prospective father that contain MHC antigens.

~~14~~ 13. A method of treating an infertility condition as in claim ~~13~~ 12 wherein the antigen is an MHC I antigen of the prospective father.

~~15~~ 14. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the one or more antigens are administered on leukocytes of the prospective father.

~~16~~ 15. A method of treating an infertility condition as in claim ~~2~~ 1 ~~wherin~~ wherein the one or more antigens are adminstrated on sperm cells of the prospective father.

~~17~~ 16. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the one or more antigens are adminstrated in the seminal plasma of the prospective father.

~~18~~ 17. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the one or more antigens are presented in purified or semi-purified form.

~~19~~ 18. A method of treating an infertility condition as in claim ~~18~~ 17 wherein the purified or semi purified one or more antigens are presented on inert or adjuvant carriers.

~~20~~ 19. A method of treating an infertility condition as in claim 2 wherein humans are being treated, and the exposure of TGFβ is to a mucosal surface and the level of TGFβ is greater than 50 ng/ml with a total dose of 150ng/ml

~~21~~ 20. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is exposed to a concentration of TGFβ of between 100 and 400ng/ml with a total dose of between 100 to 2000ng.

~~22~~ 21. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the TGFβ or derivative or analog thereofis supplied in a slow release form.

~~23~~ 22. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the exposure of the one or more antigens is to the prospective mother's genital tract in the form of the prospective father's ejaculate, and the level of exposure is determined by the cell count and antigenic density on the surface of such cells.

~~24~~ 23. A method of treating an infertility condition as in claim ~~3~~ 2 wherein humans are being treated and the one or more antigens are present on leukocytes, whereby between 10^7 and 10^9 leukocytes are adminstered to a mucosal surface.

~~25~~ 24. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the TGFβ is selected from the group of $TGF\beta_1$, $TGF\beta_2$ and $TGF\beta_3$.

~~26~~ 25. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the TGFβ is $TGF\beta_1$.

~~27~~ 26. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the TGFβ is modified.

~~28~~ 27. A method of treating an infertility condition as in claim ~~27~~ 26 wherein the modification is selected from the group comprising substitution, deletion or addition mutants, peptide fragments of TGFβ or derivative or analog thereof, and peptide fragments of TGFβ or derivative or analog thereof which have been incorporated into another protein.

~~29~~ 28. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the TGFβ or derivative or analog thereof is a member of the TGFβ superfamily.

~~30~~ 29. A method of treating an infertility condition as in claim ~~29~~ 28 wherein the member of the TGFβ superfamily is activin.

~~31~~ 30. A method of treating an infertility condition as in claim ~~2~~ 1 wherein TGFβ is administered in its active form.

~~32~~ 31. A method of treating an infertility condition as in claim ~~2~~ 1 wherein TGFβ is administered in precursor form.

~~33~~ 32. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the prospective mother is incapable of converting sufficient of the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of active TGFβ.

~~34~~ 33. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of a compound capable of activating TGFβ.

~~35~~ 34. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of plasmin, so as to increase the level of active TGFβ.

~~36~~ 35. A method of treating an infertility condition as in claim ~~2~~ 1 wherein TGFβ is administered in an unpurified form using a biological source rich in TGFβ.

~~37~~ 36. A method of treating an infertility condition as in claim ~~36~~ 35 wherein the TGFβ is administered in the form of platelets.

~~38~~ 37. A method of treating an infertility condition as in claim ~~3~~ 2 wherein humans are being treated and the exposure to TGFβ and male antigen is a multiple exposure.

~~39~~ 38. A method of treating an infertility condition as in claim ~~38~~ 37 wherein the multiple exposure is preferably performed over a period spanning at least three months prior to attempted conception.

~~40~~ 39. A method of treating an infertility condition as in claim ~~2~~ 1 wherein humans are being treated and exposure is at least one week before conception is attempted.

~~41~~ 40. A method of treating an infertility condition as in claim ~~2~~ 1 wherein the exposure is before attempted conception

~~42~~ 41. A method of treating an infertility condition as in claim ~~2~~ 1 wherein administration of TGFβ or derivative or analog thereof and the one ore more antigen occurs at least once after the prospective date of conception

~~43~~ 42. A method of treating an infertility condition as in claim ~~42~~ 41 wherein the exposure continues over a period of the first 12 weeks of pregnancy.

~~44~~ 43. A method of treating an infertility condition as in claim ~~2~~ 1 first including the step of diagnosing or testing whether the male has adequate levels of TGFβ or the female has the capacity to activate TGFβ, or alternatively whether anti-sperm antibodies exist.

~~45~~ 44. A method of treating an infertility condition as in claim ~~2~~ 1 used in conjunction with IVF treatment, whereby the transient hyporeactive immune response is elicited before transfer of the conceptus or gametes is attempted.

~~46~~ 45. A method of diagnosing an infertility condition in males by testing the level of TGFβ in seminal fluid.

~~47~~ 46. A method of diagnosing an infertility condition in a female by testing for the capacity of the female to convert the inactive form of TGFβ to the active form.

~~48~~ 47. A composition for use in treating an infertility condition, comprising TGFβ or derivative or analog thereof and one or more paternal antigens, and a pharmaceutically acceptable carrier, suitable for administration to a mucosal surface.

~~49~~ 48. A composition for use in treating an infertility condition as in claim ~~48~~ 47 wherein the composition comprises a vaginal gel.

A.P.T. PATENT AND TRADE MARK ATTORNEYS

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Your Ref:
Our Ref: 1103PCT:PJW:JWH:HJB

29 January 1999

THE COMMISSIONER OF PATENTS

WODEN ACT 2606

Sir

INTERNATIONAL Patent Application No. PCT/AU98/00149
LUMINIS PTY LTD

We refer to the Examiner's opinion of 13th October 1998 and respond thereto.

Firstly we summarise the present invention. The inventors have discovered that TGFß, which is produced in a latent form in the seminal vesicle gland and is then activated within the female reproductive tract, induces GM-CSF synthesis in uterine epithilial cells. This upregulates GM-CSF release and initiates a post-coital inflammatory response which is related to the induction of tolerance by the mother to a conceptus. Further, the inventors have shown that administration of TGFß to the female reproductive tract together with sperm or semen can elicit a tolerance towards male antigens and hence to a conceptus. Thus certain infertility conditions that may result from an inability of the female to produce tolerance to the antigens of the male may be alleviated by the practice of the present invention.

In contrast, US 5,395,825 by Feinberg, which is cited against claims 1 to 16 and 19 to 44, is concerned with the administration of TGFß to facilitate the production of fibronectin which assists implantation by promoting adhesion of the embryo to the endometrial surface. There is no disclosure or teaching of a method involving administering TGFß to elicit an immune response in the female. Following the Feinberg method, the TGFß has to be administered to coincide with the arrival of the pre-implantation embryo in the uterine cavity. We enclose herewith copies of two articles relating to TGFß. Article A is titled "Novel Delivery System for Inducing Quiescence in Intestinal Stem Cells in Rats by Transforming Growth Factor ß1" and is by Puloakkainen et. al. (Gastroenterology 1994;107;1319-1326). Article B is titled "The Enhancement of Wound Healing by Transforming Growth Factor ß1 (TGF-ß1) Depends on the Topical Delivery System" and is also by Puloakkainen et. al. (Journal of Surgical Research 1995;58;321-329). Article A, at the start of the third paragraph on page 1324, states that TGFß1 administered systemically has a half life in circulation of 5 to 7 minutes. In article B at page 324, Figure 2 shows that there is little residual TGFß remaining 3 days after release.

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The effect of TGFß on fibronectin is very short term and, given the above data regarding the lifetime of TGFß, it is clear that in the method of Feinberg the timing of the administration of TGFß has to precise so as to coincide precisely with the arrival of the pre-implantation embryo in the uterine cavity and would not at the same time be capable of eliciting an immune response. In contrast the present invention discloses a strategy wherein the administration of TGFß is carried out up to months before conception so as to promote a tolerance by the immune system to male antigens and thus improve the chances of conception. In Feinberg the TGFß is administered to the ovum or conceptus prior to introduction into the female reproductive tract. It is apparent that Feinberg does not administer paternal antigens whereas the present invention contemplates administration of TGFß and paternal antigens to the female reproductive tract or another mucosal surface. Therefore it is expected that in the present invention the TGFß is preferably administered well before attempted conception and also that much larger amounts of TGFß are administered than in the Feinberg procedure. On this basis the present invention and hence the cited claims are novel and inventive over Feinberg.

US 5,166,190 by Mather is also cited against the same set of claims. Whilst the two inventions may achieve the same end result, the methods by which the result arises out of the inventions is clearly different. Mather discloses a method for increasing fertility in males by locally administering activin so as to stimulate sperm production in males with oligospermia. The method by which activin is used in Mather is quite different to the method in which TGFß or analogue thereof is used in the present invention.

In WO 91/10445 by Woodruff the use of inhibin to increase fertility in females is disclosed. It appears that in the citation inhibin is used to induce ovulation and therefore the disclosed method does not involve the administration of one or more paternal antigens as do the claims of the present invention. Further, the two inventions are quite different-the citation discloses the use of inhibin to increase ovulation, whereas the present invention discloses the use of TGFß and one or more antigens to increase the tolerance of the female immune system to the antigens.

The Examiner has also suggested that Claim 46 is not novel in light of US 5,395,825 by Feinberg, Chu et. al. in Fertility and Sterility (66(2), 1996) and Nocera and Chu in American Journal of Reproductive Immunology (33(4), 1995). The Feinberg document discusses the use of an assay for the presence of active TGFß as a test of female fertility. The proposed assay involves measuring the levels of TGFß whereas Claim 46 of the present specification claims a method involving testing the capacity of the female to convert an inactive form of TGFß to the active form. The Chu and Nocera/Chu documents both suggest that the acidic environment of the female genital tract could activate TGFß. However the documents do not disclose a method of testing infertility based on these observations. For this reason claim 46 is novel over the citations. The inventors believe that measuring the levels of active TGFß and determining the ability of a female to convert the inactive form of TGFß to the active do not involve the same test.

Enclosed is a Statement of Amendments and retyped page 41 incorporating the amendments. A redlined copy of the claims highlighting the amendments is also enclosed.

Yours faithfully
A.P.T. Patent and Trade Mark Attorneys

Paul Wyk

Enc.

Replaced by Article 34 Amended

CLAIMS

1. A method of treating an infertility condition in a human or mammal by exposure of the prospective mother to TGFβ or an effective derivative or analog thereof before attempted conception to elicit a transient hyporesponsive immune reaction to one or more antigen of a prospective father to thereby alleviate symptoms of the infertility condition.

2. A method of treating an infertility condition as in claim 1 by exposure of a prospective mother to said one or more antigens of a prospective father and to TGFβ or an effective derivative or analog thereof before attempted conception to elicit a transient hyporesponsive immune reaction to said one or more antigen to thereby alleviate symptoms of the infertility condition.

3. A method of treating an infertility condition as in claim 2 wherein a mucosal surface of the prospective mother is exposed to the one or more antigens.

4. A method of treating an infertility condition as in claim 3 wherein the mucosal surface is selected from the group comprising an oral mucosal surface, a respiratory mucosal surface, a gastrointestinal mucosal surface or a genital mucosal surface.

5. A method of treating an infertility condition as in claim 3 wherein the mucosal surface is a genital mucosal surface.

6. A method of treating an infertility condition as in claim 3 wherein the one or more antigens and TGFβ or derivative or analog thereof is injected for systemic contact.

7. A method of treating an infertility condition as in claim 3 wherein the TGFβ or derivative or analog thereof and the one or more antigens are adminstered at one site.

8. A method of treating an infertility condition as in claim 3 wherein the TGFβ or derivative or analog thereof and the one or more antigens are each adminstered at a first site and a different site respectively.

9. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof and the one or more antigen are adminstered temporarily spaced apart.

10. A method of treating an infertility condition as in claim 9 wherein the one ore more antigens are adminstered subsequent to administration of the TGFβ or derivative or analog thereof.

11. A method of treating an infertility condition as in claim 9 wherein the one or more antigens are administered first followed by administration of TGFβ or derivative or analog thereof.

12. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are chosen as a result of being particularly antigenic and prominent either on the sperm, or on the conceptus.

13. A method of treating an infertility condition as in claim 2 wherien the one or more antigens are present on cells taken from the prospective father that contain MHC antigens.

14. A method of treating an infertility condition as in claim 13 wherein the antigen is an MHC I antigen of the prospective father.

15. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are administered on leukocytes of the prospective father.

16. A method of treating an infertility condition as in claim 2 wherin the one or more antigens are adminstrated on sperm cells of the prospective father.

17. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are adminstrated in the seminal plasma of the prospective father.

18. A method of treating an infertility condition as in claim 2 wherein the one or more antigens are presented in purified or semi-purified form.

19. A method of treating an infertility condition as in claim 18 wherein the purified or semi purified one or more antigens are presented on inert or adjuvant carriers.

20. A method of treating an infertility condition as in claim 2 wherein humans are being treated, and the exposure of TGFβ is to a mucosal surface and the level of TGFβ is greater than 50 ng/ml with a total dose of 150ng/ml.

31. A method of treating an infertility condition as in claim 2 wherein TGFβ is administered in its active form.

32. A method of treating an infertility condition as in claim 2 wherein TGFβ is administered in precursor form.

33. A method of treating an infertility condition as in claim 2 wherein the prospective mother is incapable of converting sufficient of the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of active TGFβ.

34. A method of treating an infertility condition as in claim 2 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of a compound capable of activating TGFβ.

35. A method of treating an infertility condition as in claim 2 wherein the prospective mother is incapable of converting the inactive form of TGFβ to active TGFβ, and the method of treating includes administration of plasmin, so as to increase the level of active TGFβ.

36. A method of treating an infertility condition as in claim 2 wherein TGFβ is administered in an unpurified form using a biological source rich in TGFβ.

37. A method of treating an infertility condition as in claim 36 wherein the TGFβ is administered in the form of platelets.

38. A method of treating an infertility condition as in claim 3 wherein humans are being treated and the exposure to TGFβ and male antigen is a multiple exposure.

39. A method of treating an infertility condition as in claim 38 wherein the multiple exposure is preferably performed over a period spanning at least three months prior to attempted conception.

40. A method of treating an infertility condition as in claim 2 wherein humans are being treated and exposure is at least one week before conception is attempted.

41. A method of treating an infertility condition as in claim 2 wherein the exposure is before attempted conception.

42. A method of treating an infertility condition as in claim 2 wherein administration of TGFβ or derivative or analog thereof and the one ore more antigen occurs at least once after the prospective date of conception.

43. A method of treating an infertility condition as in claim 42 wherein the exposure continues over a period of the first 12 weeks of pregnancy.

44. A method of treating an infertility condition as in claim 2 first including the step of diagnosing or testing whether the male has adequate levels of TGFβ or the female has the capacity to activate TGFβ, or alternatively whether anti-sperm antibodies exist.

45. A method of treating an infertility condition as in claim 2 used in conjunction with IVF treatment, whereby the transient hyporeactive immune response is elicited before transfer of the conceptus or gametes is attempted.

46. A method of diagnosing an infertility condition in males by testing the level of TGFβ in seminal fluid.

47. A method of diagnosing an infertility condition in a female by testing for the capacity of the female to convert the inactive form of TGFβ to the active form.

48. A composition for use in treating an infertility condition, comprising TGFβ or derivative or analog thereof and one or more paternal antigens, and a pharmaceutically acceptable carrier, suitable for administration to a mucosal surface.

49. A composition for use in treating an infertility condition as in claim 48 wherein the composition comprises a vaginal gel.

21. A method of treating an infertility condition as in claim 2 wherein the mucosal surface is exposed to a concentration of TGFβ of between 100 and 400ng/ml with a total dose of between 100 to 2000ng.

22. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereofis supplied in a slow release form.

23. A method of treating an infertility condition as in claim 2 wherein the exposure of the one or more antigens is to the prospective mother's genital tract in the form of the prospective father's ejaculate, and the level of exposure is determined by the cell count and antigenic density on the surface of such cells.

24. A method of treating an infertility condition as in claim 3 wherein humans are being treated and the one or more antigens are present on leukocytes, whereby between 10^7 and 10^9 leukocytes are adminstered to a mucosal surface.

25. A method of treating an infertility condition as in claim 2 wherein the TGFβ is selected from the group of $TGF\beta_1$, $TGF\beta_2$ and $TGF\beta_3$.

26. A method of treating an infertility condition as in claim 2 wherein the TGFβ is $TGF\beta_1$.

27. A method of treating an infertility condition as in claim 2 wherein the TGFβ is modified.

28. A method of treating an infertility condition as in claim 27 wherein the modification is selected from the group comprising substitution, deletion or addition mutants, peptide fragments of TGFβ or derivative or analog thereof, and peptide fragments of TGFβ or derivative or analog thereof which have been incorporated into another protein.

29. A method of treating an infertility condition as in claim 2 wherein the TGFβ or derivative or analog thereof is a member of the TGFβ superfamily.

30. A method of treating an infertility condition as in claim 29 wherein the member of the TGFβ superfamily is activin.

29 January 1999

Patent Cooperation Treaty Application No. PCT/AU98/00149
LUMINIS PTY LTD

STATEMENT OF AMENDMENTS

4. Cancel Page 41 on file containing claims 41 to 48 and replace with amended Page 41 containing Claims 41 to 48 wherein amendments have been made to Claim 47. The remaining claims are unchanged. A redlined copy of the claims showing the amendments is also included.

41. A method of treating an infertility condition as in claim 1 wherein administration of TGFβ or derivative or analog thereof and the one ore more antigen occurs at least once after the prospective date of conception

42. A method of treating an infertility condition as in claim 41 wherein the exposure continues over a period of the first 12 weeks of pregnancy.

43. A method of treating an infertility condition as in claim 1 first including the step of diagnosing or testing whether the male has adequate levels of TGFβ or the female has the capacity to activate TGFβ, or alternatively whether anti-sperm antibodies exist.

44. A method of treating an infertility condition as in claim 1 used in conjunction with IVF treatment, whereby the transient hyporeactive immune response is elicited before transfer of the conceptus or gametes is attempted.

45. A method of diagnosing an infertility condition in males by testing the level of TGFβ in seminal fluid.

46. A method of diagnosing an infertility condition in a female by testing for the capacity of the female to convert the inactive form of TGFβ to the active form.

47. A composition for use in treating an infertility condition, comprising substantially purified TGFβ or derivative or analog thereof and one or more paternal antigens, and a pharmaceutically acceptable carrier, suitable for administration to a mucosal surface.

48. A composition for use in treating an infertility condition as in claim 47 wherein the composition comprises a vaginal gel.